# Musculoskeletal System

The development of the musculoskeletal system is complex, requiring the coordinated integration of mesenchymal derivatives from different parts of the embryo and a variety of epithelial/mesenchymal interaction. Three distinct subpopulations of mesenchyme produce the majority of the system.

- *Paraxial mesenchyme* gives rise to the striated muscle throughout the head, trunk and limbs, virtually exclusively via the somites or preoccipital somitomeres (although axial mesenchyme from the prechordal plate produces the extrinsic eye muscles).
- *Somatopleuric mesenchyme* and a discrete portion of each somite, in the main, give rise to the skeletal elements, ligaments, tendons, fasciae, muscular and dermal connective tissue throughout the trunk and limbs. The former also patterns the development of the nerves, muscles and blood vessels in these locations.
- *Neural crest mesenchyme* produces the skeletal elements of the viscerocranium and much of the neurocranium, the ligaments, tendons, fasciae and muscular connective tissue throughout the head, including the meninges and dermal connective tissues. The neural crest also patterns the development of the nerves, muscles and blood vessels in these locations.

Germinal epithelia, which provide the populations of mesenchyme cells for these fates, are generated locally in the somites, each of which provides a discrete germinal epithelial plate for the production of myoblasts, and more extensively in the proliferating somatopleuric mesothelium. Neural crest cells proliferate as they migrate and also in situ. In all cases epithelial tissue close to the mesenchyme, often specifically ectoderm, contributes to the developmental processes by initiating some differentiation pathways and preventing others.

Because of the diversity of cell populations which contribute to the musculoskeletal system, its development will be considered in the following order:

**(1) Development of the Axial Structures**

—development of the musculoskeletal tissues of the trunk, i.e. the vertebral column and associated muscles. These structures are formed by the paraxial mesenchyme which surrounds the neural tube and notochord, and laterally by somatopleuric mesenchyme.

—development of the musculoskeletal tissues of the head, i.e. the skull and associated muscles. These structures are formed by several mesenchymal populations, i.e. a specialized portion of the axial musculoskeletal tissue, a mesenchymal population from the prechordal plate and a significant mesenchymal population from the ectodermal neural crest.

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## (2) Development of the Appendicular Structures

The musculoskeletal tissues of the limbs are formed from both somatopleuric mesenchyme and paraxial mesenchyme.

Our understanding of the general development of the connective and muscular tissues in the skeletal system has improved significantly with the advances in molecular biology and it is possible to see common developmental pathways which are followed by all myoblasts, chondroblasts or fibroblasts, etc. regardless of their site of origin. A brief account of some of these basic mechanisms may assist the interpretation of more specific events.

### *General Development of Connective Tissue Cells*

The most fundamental facet of connective tissue differentiation is the production of mesenchymal condensations which, according to Atchley and Hall (1991), are the basic units from which morphology is constructed during development. Five developmental criteria identify a condensation:

- the number of stem cells
- the time of condensation initiation
- the mitotically active fraction
- the rate of cell division
- the rate of cell death.

These criteria may vary individually or in concert producing variability in developmental processes. A condensation is the first cellular product of epithelial/mesenchymal tissue interactions. (For a general account of epithelial/mesenchymal interactions, see p. 110.) The formation of a mesenchymal condensation is associated with formation of gap junctions that allow intercellular communication followed by production of extracellular matrix molecules, if sufficient cells are associated within a condensation (Hall & Miyake 1992). The type and quantity of the matrix can induce and maintain production of further matrix molecules by competent cells. Particular matrix molecules are associated with specific developmental lineages and can be used to distinguish different cell fates, for example an osteogenic fate from a chondrogenic.

It is not yet clear how cells are committed to a connective tissue lineage; however, it has been shown that single mesenchyme cells will differentiate into chondroblasts if they are maintained in a rounded configuration. Connective tissue develops from mesenchyme of different origins, for example from somatopleuric mesenchyme, cephalic neural crest cells and parts of the somite (splanchnopleuric mesenchyme also in association with the viscera). The formation of cartilage has been extensively studied; however, less is known about the development of the widespread

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range of connective tissue or the origin of the osteoblastic lineage. Chondrogenesis is generally initiated from mesenchyme in response to an extracellular matrix mediated interaction, either via a basal lamina as in the sclerotomes (see below), or via an ectodermal mesenchymal interaction as in the limbs and facial processes (see below). Sclerotomal cells are already determined to a chondrogenic lineage, perhaps even before somite formation; interaction with the basal laminae of the notochord and neural tube enhances the differentiation process. The mesenchyme of the limb requires both the presence of an ectodermal sleeve early in development and then subsequent interaction with extracellular matrix products for both chondrogenic and fibrocyte differentiation. A high cell density in the core of the limb is required for chondrogenic differentiation whilst an antichondrogenic zone immediately beneath the ectoderm seems to prevent the differentiation of cartilage within the dermis and myogenic zone. Limb buds cultured in the coelomic cavity usually chondrify in their peripheral zones where the ectoderm is lacking or replaced by another kind of epithelium (Brand et al 1985). The ectoderm is believed to produce matrix molecules which encourage cell flattening and fibrogenic differentiation (Christ et al 1986). Expression of type II collagen in mesenchyme cells is often a sign of terminal differentiation along a chondrogenic lineage. The ultimate fate of such cells is production of type X collagen; when this occurs the cells hypertrophy and will ultimately die. Hypertrophied cells can start the mineralization process within the expanded cartilage lacunae. Regions of persistent cartilage, (e.g. trachea, pinna, etc.) do not permit the final differentiation fate of the cell line.

The factors promoting osteoblast development are not clear. Osteogenesis coincides with the vascularization of either a cartilage model, as in *endochondral ossification*, or of a mesenchymal condensation directly, as in *intramembranous ossification*. Ossification occurs at a much slower rate than chondrogenesis (for a fuller description of these processes see p. 471). Chondroclasts and osteoclasts have been identified in older developing limbs remodelling developing bone and cartilage; they may represent the same cell line in different locations (Jacob at al 1986). Adipocytes are also related to chondroblasts and osteoblasts and fibroblasts.

### General Development of Skeletal Muscle

A myogenic lineage, noted by the expression of myogenic determination factors, can be demonstrated transitorily in some cells shortly after their ingression through the primitive streak. The skeletal muscle found throughout the body is derived from the paraxial mesenchyme which segments to form the somites (see also development of the extrinsic ocular muscles).

Cells committed to a myogenic lineage will undergo a series of proliferative mitotic divisions prior to passage through a *terminal division* resulting in their restriction as *postmitotic myoblasts*. Postmitotic myoblasts can begin to transcribe the mRNAs for the major contractile proteins *actin* and *myosin* as well as a number of regulatory proteins of muscle contraction. Finally postmitotic myoblasts will assume a characteristic spindle shape and begin to fuse with one another, creating a tube-like *syncytium*, the *myotube*. (Interestingly myoblasts from different vertebrates will fuse to form hybrid myotubes.) Subsequent to fusion, *myofibrils* assemble in the periphery of the myotube. The early myofibrils develop the cross-striated organization first at the Z line, an α-actinin rich structure that anchors the actin filaments to form the I-Z-I complexes, and later in the A band region, occupied by the myosin filaments. Sarcomere formation proceeds from the

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periphery towards the centre of the myotubes. When this process is complete, the nuclei migrate from the centre to the periphery, and the syncytium is now called a *myofibre*. Myofibrils align laterally with one another, the sarcoplasmic reticulum and T-tubules become arranged in transverse orientation, and the myofibre continues to grow by splitting of myofibrils as well as by addition of new myofibrils.

During development at least three populations of myoblast are formed. Embryonic myoblasts give rise to primary myotubes and muscle fibres, and thus embryonic muscle. Subsequently smaller, secondary myotubes and muscle fibres arise from late myoblasts. Finally satellite cells which are also contained within the basal lamina differentiate. These latter cells may divide in postnatal life to provide new myoblasts to fuse with the muscle fibres ensuring growth of the muscle. For more information on subsets of muscle fibres consult Section 7.

The development of the central nervous system is crucial for normal formation of the *fetal* myoblast lineage. Formation of secondary fibres appears to be nerve dependent; the number of secondary fibres is reduced by denervation. It is suggested that secondary myotubes are initiated only at sites of innervation of primary myotubes.

## Axial Skeleton and Muscles

### *Somitogenesis*

Cells destined to become paraxial mesenchyme ingress through the lateral aspect of the primitive node and rostral primitive streak (**3.42**); the mesenchyme cells thus formed retain contact with both the epiblast and hypoblast basal laminae as they migrate to their paraxial position and this persists for some time after reaching their destination.

After the onset of neurulation the paraxial mesenchyme caudal to the otic vesicle undergoes segmentation (**3.131**) in a craniocaudal progression forming discrete clusters of mesenchyme cells; this stage is termed *compaction*. In each tight cluster of paraxial mesenchyme the cells re-establish juxtaluminal junctions and organize themselves into an *epithelial somite*. The cells of the epithelial somite are polarized with respect to a central lumen which contains some mesenchymal core cells. The Golgi apparatus and mitotic figures are located in the apical region of the cells, as are actin and α-actinin; cilia develop on the free surface. The cells are joined by tight junctions and the basal surface rests on a basal lamina containing collagen, laminin, fibronectin and cytotactin (Keynes & Stern 1988). Processes from the somite cells pass through this basal lamina to contact the basal laminae of the neural tube and notochord (Hay 1968). A variety of cell adhesion molecules have been demonstrated in epithelial somites. It is worth noting that a single somite can be described as having six faces, like a cube; it is now apparent that each facet has a slightly different fate. Further, the position along the embryo may alter the developmental fate of parts of the somite.

The epithelial somite undergoes rapid development in the following manner: the cells of the

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ventromedial wall seem to be pulled towards the notochord, and despite extensive juxtaluminal junctions the cells break apart. The newly formed mesenchymal cells, collectively termed the *sclerotome*, migrate medially towards the notochord; they will give rise to the bones, joints and ligaments of the vertebral column (see below). The remaining cells of the somite are now termed the *epithelial plate of the somite* or the *dermomyotome*. This epithelium produces the cell lines which will give rise to (nearly) all the striated muscles of the body. Three separate myogenic lines can be seen. Firstly, cells produced along the *craniomedial edge of the epithelial plate* elongate from the cranial to the caudal edge on the underside of the basal lamina of the plate; they are collectively termed the *myotome*. (The latter term was previously used to describe *all* the muscle forming cells of the somite; now it is usually restricted to cells deriving from the craniomedial edge.) They will give rise to the skeletal muscle dorsal to the vertebrae, the epaxial musculature (see below). Secondly, after initiation of the myotome, cells produced from the *ventrolateral edge of the epithelial plate*, opposite the limb bud, migrate into the developing limb to give rise to its skeletal muscle (see below). Lastly, the *remaining epithelial plate* (and underlying myotome cells) *grows into the flank region of the body*. The epithelial plate is still proliferating at the beginning of this stage. Later the epithelial plate cells revert to mesenchyme and processes from contiguous somites fuse to form a unified premuscular mass which gives rise to the ventrolateral muscles of the body wall (see below).

It was, for long, the case that once the myotome cells could be identified the remaining epithelial plate was termed the dermatome, its fate being described as forming the dermis of the skin. However, it is now clear that the epithelial plate continues to provide a significant source of myogenic precursor cells as it elongates into the body wall. Thus the detailed intimate relationship between the epithelial plate/dermatome, the generation of myogenic cells and the patterning of the epidermis of the skin is, as yet, by no means clear (see later). Studies describing a somitic contribution to the dermis, from the dermatome, localize it to the dermis over the epaxial muscles (Christ et al 1983), a much smaller distribution than the segmental portion of skin usually implied by this term.

The rate of somite formation has been estimated at approximately one pair every 3 hours (Menkes et al 1961; Chernoff & Lash 1981). The regularity of somite formation provides criteria for staging embryos; staging schemes have been developed both by Lash and Ostrovsky (1986) and by Ordahl (1993). Lash and Ostrosky describe five stages, from somitomere identification (see above) to the production of an epithelial somite distinct from the presegmental plate. Ordahl notes that morphogenetic events occur in successive somites at approximately the same rate. He designates the somite most recently formed from the segmental plate (stage 5 of Lash & Ostrovsky) as stage I, the next most recent as stage II, etc. After the embryo forms an additional somite, the ages of the previously formed somites increase by one roman numeral. In this conceptualization of somitogenesis compaction occurs at stage I; epithelialization at stages II to III; formation of mesenchymal sclerotome cells from stage V; myotome formation at stage VI; early migration of the ventrolateral lip of the epithelial plate and production of myotome cells can still be seen at stage X.

Differences have been identified in the fates of each of the six facets of a somite. Firstly the medial and lateral halves of the early epithelial somite have different origins and fates, later the

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cranial and caudal halves of the epithelial plate differ and finally the cranial and caudal halves of the sclerotome have different properties and fates. Experimental studies have shown that the precise developmental fates of these portions of the somite may be prescribed as the precursor cells ingress from the epiblast. Compaction and epithelialization may then shuffle these cells into their appropriate positions in the somite prior to migration.

Selleck and Stern (1991) have shown that the medial halves of somites are formed from cells migrating through the lateral portion of Hensen's node; the lateral halves derive from ingression through the primitive streak approximately 200 μm caudal to the node. The two somite halves do not seem to intermingle. The *medial half of the somite* produces both the *sclerotome* and the *myotome* (*epaxial musculature*); the *lateral half of the somite* provides the *hypaxial* and *limb musculature* (Ordahl & Le Douarin 1992; Ordahl 1993). (Interestingly the innervations of these muscle groups are provided by the posterior ramus of a spinal nerve for the epaxial muscles, and the ventral ramus for the hypaxial and limb muscles.) Of the other facets, the cranial portion of the epithelial plate is the site of origin of the myotome (see below). Differences in the craniocaudal fates of the portions of the somites have been studied in the development of the sclerotomes (see below).

### *Formation of the Vertebrae From Sclerotomes*

The sclerotome forms from the ventromedial border of the epithelial somite. An *intrasegmental boundary* (fissure or cleft) appears within the *sclerotome* dividing it into loosely packed *cranial* and densely packed *caudal* halves; this boundary is initially filled with extracellular matrix and only few cells. The epithelial plate and later the myotome spans the two half-sclerotomes. The sclerotomal cells migrate towards the notochord which they surround, and with which they undergo a matrix-mediated interaction, differentiating chondrogenetically to form the cartilaginous precursor of the vertebral body. The perinotochordal sheath transiently expresses type II collagen which is believed to initiate type II collagen expression, and thereafter a chondrogenic fate, in those mesenchyme cells which contact it (see also development of the chondrocranium, p. 274). Each *vertebra* is formed by the combination of much of the *caudal half of one bilateral pair of sclerotomes with much of the cranial half of the next caudal pair of sclerotomes.* Their fusion around the notochord produces the blastemal centrum of the vertebra (**3.132**; see also **3.134**). The mesenchyme adjoining the intrasegmental sclerotomic fissure now increases greatly in density forming a well-defined *perichordal disc*. This intervenes between the successive centra of two vertebrae and is the future annulus fibrosis of the intervertebral symphysis ('disc').

The boundary of the head and neck corresponds to the boundary between the 5th and 6th somites. The first true somite disappears early and somites 2–5 (occipital 1–4) fuse to form the basioccipital bone (see, however, preoccipital somitomeres). The vertebrae are formed from the 6th somite caudally, C1 being formed by the caudal half of occipital somite 4 and the cranial half of cervical somite 1 (**3.132**, **133**). This shift of somite number and vertebral number accounts for the production of seven cervical vertebrae from eight cervical somites.

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The basic pattern of a typical vertebra is initiated by this recombination of caudal and cranial sclerotome halves, followed by differential growth and sculpturing of the sclerotomal mesenchyme which encases the notochord and neural tube. This is the blastemal stage of vertebral development (**3.134**). As noted, the *centrum* encloses the notochord and lies ventral to the neural tube. From the dorsolateral angles of the centrum the neural arch curves to enclose the neural tube; from the zones of neurocentral confluence the arch comprises paired bilateral pedicles (ventrolaterally) and laminae (dorsolaterally) which coalesce in the midline dorsal to the neural tube. From the latter arises the anlage of the vertebral spine. On each side three further processes are delineated, projecting from the junction of pedicle and lamina cranially, caudally and laterally. The cranial and caudal projections are the blastemal *articular processes* (zygapophyses) and these become contiguous with reciprocal processes of adjacent vertebrae, their junctional zones the future zygapophyseal joints. The lateral projections are the true vertebral *transverse processes* (see below). Finally, growing anterolaterally from the ventral part of the pedicles, i.e. near the centrum, from the neighbouring perichordal disc, and, at most thoracic levels, with accessions from the next caudal adjacent pedicles, bilateral *costal processes* develop; these expand to meet the tips of the transverse processes. Note that the definitive vertebral *body* is compound, a median centrum and ventral expanded pedicle ends (bilateral) dorsolaterally.

In *cervical vertebrae* (**3.134**) the transverse process is dorsomedial to the foramen transversarium, while the costal process, corresponding to the head, neck and tubercle of a rib, limits the foramen ventrolaterally and dorsolaterally. The distal parts of these cervical costal processes do not normally develop; occasionally they do so in the case of the seventh cervical vertebra, even developing costovertebral joints. Such *cervical ribs* may reach the sternum (p. 268). In the thoracic region the *thoracic costal processes* attain their maximum length as *the ribs* (see below). The extent of the transverse and costal processes of each vertebra can be compared in (**3.134**).

The *type* of vertebra is specified very early in development. If a group of thoracic somites is transplanted to the cervical region, ribs will still develop (Kieny et al 1972; Goldstein & Kalcheim 1992). Interestingly it is the sclerotome which is restricted; the myotome will produce muscle characteristic of the new location. At present the exact contribution of the caudal and rostral parts of the sclerotomes to the neural arches, pedicles and laminae and articular and transverse processes are not yet entirely clear (Bagnall et al 1988; Goldstein & Kalcheim 1992); similarly the exact origin of the intervertebral disc has not been established.

Condensation of the sclerotomal mesenchyme around the notochord can be seen in stage 15 human embryos as can right and left neural processes. Chondrification begins at stage 17, initiating the cartilaginous stage (**3.134**). Each centrum chondrifies from one cartilage anlage (Uhthoff 1990). Each half of a neural arch is chondrified from a centre starting in its base and extending ventrally into the pedicles, to meet, expand and blend with the centrum, and dorsally into the laminae. By stage 23 there are 33 or 34 cartilaginous vertebrae; however, the spinous processes have not yet developed giving a general appearance of total spina bifida occulta. Fusion of the spines does not occur until the fourth month. The transverse and articular processes

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are chondrified in continuity with the neural arches; intervening zones of mesenchyme which do not become cartilage mark the sites of their interarticular (zygapophyseal) joints and the complex of costovertebral joints, and synovial cavities appear later in these.

In general the thoracic spine develops ahead of the cervical and lumbar spine; however, towards the end of the second month ossification commences in the cartilaginous vertebrae in a craniocaudal progression. After 16 weeks it has progressed to L5 and ossification of each additional vertebra occurs over a period of 2–3 weeks with S2 being ossified by 22 weeks. In most cases S1 can be located at the level of the top of the iliac crest (Budorick 1991). Further details of ossification are described elsewhere (p. 471).

## Intervertebral Discs

Whereas the sclerotomal mesenchyme forming the body of the vertebrae replaces the notochordal tissue which it surrounds, between the developing vertebrae the notochord expands as localized aggregates of cells and matrix, thus forming the *nucleus pulposus* of the intervertebral disc (**3.132**, **134**). This nucleus is surrounded by the intermediate part of each *perichordal disc* which forms the annulus fibrosus and differentiates into an external laminated fibrous zone and an internal cuff around the nucleus pulposus. The inner zone contributes to the growth of the outer, and near the end of the second month of embryonic life it begins to merge with the notochordal tissue, being ultimately converted into fibrocartilage. After the sixth month of fetal life notochordal cells in the nucleus pulposus degenerate, being replaced by cells from the internal zone of the annulus fibrosus. This degeneration continues until the second decade of life, by which time all the notochordal cells have disappeared (p. 513). Thus, in the adult, notochordal vestiges are limited, at the most, to non-cellular matrix.

It is to be re-emphasized here that the original sclerotomes are coextensive with the individual *metameric body segments*, and that each sclerotomic fissure, perichordal disc, and finally the maturing intervertebral disc lies opposite the *centre* of each *fundamental body segment*. From this, it follows that the discs also correspond in level to (i.e. form the anterior boundary of) the intervertebral foramina, their contained mixed spinal nerves, ganglia, vessels and sheaths. Posteriorly, bounding the foramina are the capsules of the synovial interarticular (zygapophyseal) joints. Cranially and caudally lie the rims of the vertebral notches of adjacent vertebrae. Thus all the structures listed (and other associated ones) are often designated *segmental*, whereas because of their mode of development, vertebral bodies are designated *intersegmental*.

## *Ribs, Costal Cartilages and Sternum*

## Ribs

These develop from the costal processes of the primitive vertebral arches, extending between the myotomic muscle plates. The development of ribs is usually limited to the thoracic vertebrae although ribs can arise occasionally from the seventh cervical vertebra. In the thoracic region

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(**3.134**) costal processes grow laterally to form a series of *precartilaginous ribs*. The transverse processes grow laterally behind the vertebral ends of the costal processes, at first connected by mesenchyme which later becomes differentiated into the ligaments and other tissues of the costotransverse joints. The capitular costovertebral joints are similarly formed from mesenchyme between the proximal end of the costal processes and the perichordal disc, and adjacent neural arch derived parts of usually two (sometimes one) vertebral bodies. Ribs 1–7 (vertebrosternal) curve round the body wall to reach the developing sternal plates. Ribs 8–10 (vertebrochondral) are progressively more oblique and shorter, only reaching the costal cartilage of the rib above and contributing to the costal margin. Ribs 11 and 12 are free (floating), with cone-shaped terminal cartilages providing muscle attachments (see p. 541). In *lumbar vertebrae* (**3.134**) the costal processes do not develop distally, but their proximal parts become the 'transverse processes' of these vertebrae, whose morphologically *true* transverse processes may be represented by their accessory processes (p. 526). Occasionally, movable ribs may develop in association with the first lumbar vertebra. Only the upper two or three *sacral costal processes* usually develop significantly (**3.134**). They fuse into the lateral mass of the sacrum, forming its ventral part. The *coccygeal vertebrae* are apparently devoid of costal processes.

## Sternum

This is formed from bilateral condensations of *somatopleuric mesenchyme* (Gumpel-Pinot 1984) immediately ventral to the primordia of the clavicles and ribs; these are termed the sternal plates. They are immediately ventral to the rudiments of the clavicles and ribs, but are independent of them in their formation. As the ribs lengthen, the sternal plates *chondrify* and move medially towards each other fusing across the midline in a craniocaudal direction. This forms the *manubrium sterni* and four *sternebrae* which form the sternal body with which the clavicles and upper seven pairs of costal cartilages establish contact. The *xiphoid process* develops as a caudal extension of the sternal body. Hypertrophy of the cartilage cells as a preliminary to ossification occurs opposite future intercostal spaces. The ossification and further growth of the sternum and ribs is described later (see **6.123**, and p. 539).

## *Formation of the Axial Muscles From Myotomes*

Myogenic determination factors, MyoD, myogenin, Myf 5 and herculin/MRF 4 can first be detected in the medial half of the somite as early as stage II (Ordahl 1993), several hours prior to the onset of myotome formation. The *myotome* is formed in the following manner: cells of the epithelial plate are mainly orientated perpendicular to the back; however, the cells have different orientations according to their positions: they are transversely orientated along the dorsomedial edge and longitudinally orientated within the cranial edge of the epithelial plate (**3.131**). Myotome cells originate from the longitudinally orientated cells at the cranial edge of the epithelial plate. Individual cells are originally produced and anchored at the craniomedial corner of the epithelial plate. Subsequently each sends a process to the caudal edge of the plate where it forms a second anchor point. Thus myotome formation continues caudally along the dorsomedial edge and laterally along the cranial edge. Each mononucleated, myotome cell thus becomes very elongated perpendicular to the cells of the epithelial plate. Development of subsequent cells

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produces a triangular shape of the myotome in its early formation. The growing myotome first reaches the caudal somite border on the medial side and later the ventrolateral edge (Kaehn et al 1988). When the vertebral bodies form, the future intervertebral fissure divides the sclerotome into rostral and caudal halves leaving the myotome fibres spanning the intervertebral joints and foramina. Thus the myotome derived muscles are always in a position to move adjacent vertebrae relative to each other.

Myotome cells are all *postmitotic embryonic myoblasts*; they fuse to form syncytia later in development (see above) to produce the *epaxial* musculature, the skeletal muscle dorsal to the vertebrae (erector spinae). The normal development of these myoblasts requires the presence of the neural tube. It is also suggested that there is a possible interaction between precursor myotome cells and the medial neural crest cells which are commencing their migration at this time. The epaxial muscles are innervated by the dorsal ramus of each spinal nerve. The latter divides into its primary dorsal and ventral rami as it emerges from its intervertebral foramen (see above).

At much later stages *satellite cells* enter the myotome. Interestingly the development of endo-, peri- and epimysium in relation to the epaxial muscles has not been addressed; no population of connective tissue mesenchyme has yet been identified with this body region.

## Ventrolateral Trunk Muscles

These are formed from the epithelial plate of the somite. After production of the myotome and the precursor myogenic cells of the limb, the remaining epithelial plate (and attached myotome) grows into the *flank somatopleuric mesenchyme*. At this stage the epithelial plate is still proliferating and producing myogenic precursor cells. The epithelial plate has a leading edge or process from which single cells or clusters of cells migrate in a ventral direction. It may be that these epithelial plate cells, which are in a more immature state of differentiation, act as *pioneer cells* for further cell movement (Jacob et al 1986). The previously segmented processes from adjacent epithelial plates form a *unified premuscular mass*. Both postmitotic myoblast cells and still dividing plate cells can be seen in the body wall; this may represent early and later forming myoblasts which will form heterokaryotic myotubes.

The *premuscular mass* subdivides into *abdominal muscle blastemata* for the *external* and *internal oblique* muscles, *transversus abdominis* and *rectus abdominis*. At this time the number of somatopleural fibroblasts situated within the muscle-forming zone increases, and myotubes can be first seen. Lastly, there is a ventral shift of the already separated muscle blastemata within the growing abdominal wall to their definitive positions. During this process muscle differentiation continues and muscular connective tissue, tendons and aponeuroses develop.

## The Diaphragm

This is a partition between the thoracic and abdominal cavities; it derives from a variety of mesenchymal populations. Ventrally the *septum transversum mesenchyme* anchors the

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diaphragm to the anterior abdominal wall where it attaches to the xiphisternum and costal margin. The central portion of the diaphragm is formed from *splanchnopleuric mesenchyme* which surrounds the oesophagus and inferior vena cava and coats the pleuroperitoneal membranes. Laterally the diaphragm derives from the *somatopleuric mesenchyme* which is excavated by extension of the secondary pleural cavities into the costodiaphragmatic recesses. *Somitic myocytes* from the ventrolateral edge of the epithelial plate of somites C3, 4 and 5 migrate into the lateral regions of the diaphragm including the somatopleuric part. The central region becomes tendinous. The posterior attachment of the diaphragm descends to lower and lower positions until at the end of the second month it is opposite T12 or L1 (**3.135**, **136**; see also p. 181).

### Pelvic Floor

This consists of the *ligamentous supports of the cervix*, and the *pelvic* and *urogenital diaphragms*, and constitutes another partition which traverses the body cavity. The dimensions of the pelvic cavity are much smaller than those at the caudal end of the thorax and the pelvic diaphragm is thus a smaller structure. Because of the irregular shape of the innominate bones the pelvic outlet has two planes which are filled by muscular groups arranged at different levels and directions. There is little information available about pelvic floor development in the human. The striated muscle derives from the somitic epithelial plates in a similar manner to the ventrolateral body wall. The *puborectalis* muscle appears in 20–30 mm embryos, following opening of the anal membrane, and striated muscle fibres can be seen at 15 weeks (Bourdelat 1992). Also at this time the smooth muscle of the urethral sphincter can be seen.

### Upper and Lower Ends of the Trunk

The upper and lower ends of the trunk are narrowed as a result of the development of axial structures cranially, and both axial and appendicular structures caudally. Cranially, the size of the ribs, the cervical pleura with its suprapleural membrane and the attached scalenus minimus muscle, together with the disposition of the other scaleni, create a narrow thoracic inlet which admits to the thorax only the contents of the root of the neck. Caudally, however, the pelvic outlet serves a dual function. It maintains the position of the pelvic organs and continence of the excretory organs by an arrangement of sphincter muscles. In addition, particularly with reference to the size of the human fetal head at term, the osteoligamentous boundary of the pelvic outlet, in the human, is relatively larger than that of all other quadrupeds; thus it requires a relatively extensive muscular and fibrous diaphragm.

## Head

The head is composed of the skull surrounding the brain, and an outer covering of muscles, glands and skin. The skull has two distinct portions: that surrounding the brain and special sense organs—the *neurocranium*—and the lower face and jaws (also the palate, hyoid, epiglottis and larynx)—the *viscerocranium*. Each part derives from different mesenchymal populations and by different methods. The neurocranium develops from the *paraxial mesenchyme* in the head, i.e.

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the first five somites and the unsegmented somitomeres rostral to the first somite (Meier 1981), and from ectoderm via the *neural crest*. The basal portion of the skull is similar in structure and development to the vertebral column and is preformed in cartilage. The viscerocranium derives from ectoderm via invaginated head *neural crest* which streams into the developing arches forming all the connective tissue elements of the face. Bones of the viscerocranium form in the main from membranous ossification but there are cartilage models in each arch. The contribution of neural crest to the neurocranium in mammals is not yet clear, although it has been established in the chick that neural crest mesenchyme gives rise to the large bones lateral and dorsal to the brain by membranous ossification (Couly et al 1992). Lateral plate mesenchyme does not extend into the head (see p. 286).

## Neurocranium

The bones of the skull (**3.137**) are developed in the mesenchyme which surrounds the cerebral vesicles but, before the osseous state is reached, the cranium passes through blastemal and cartilaginous stages like other parts of the skeleton. However, not all parts pass through a phase of chondrification; and hence the *chondrocranium* is incomplete, the remainder comprising the mesenchymatous, *blastemal desmocranium*. Most of the cranial vault and limited parts of its base are thus not preformed in cartilage. The mesenchymatous (membranous) and cartilaginous parts of the skull will, for convenience, be considered in sequence; they develop together and complement each other in forming the complete cranium, some of whose bones are composite structures derived from both sources. All elements, of course, pass first through a mesenchymatous phase (**3.137**).

The *blastemal skull* (desmocranium) begins to appear at the end of the first month as a condensation and thickening of the mesenchyme which surrounds the developing brain, forming localized masses which are the earliest distinguishable cranial elements. The first masses evident are in the occipital region, outlining the basilar (ventral) part of the occipital bone. These form an *occipital plate*, from which two extensions on each side grow laterally and spread to complete a foramen around each hypoglossal nerve. At the same time the mesenchymal condensation extends forwards, dorsal to the pharynx, to reach the primordium of the hypophysis, thus establishing the *clivus* of the cranial base and the *dorsum sellae* of the future sphenoid bone. Early in the second month it surrounds the developing stalk of the hypophysis and extends ventrally and rostrally between the two halves of the nasal cavity, where it forms the anlage of the ethmoid bone and of the nasal septum. The notochord traverses the ventral occipital plate obliquely, being at first near its dorsal surface and then lying ventrally, where it comes into close relationship to the epithelium of the dorsal wall of the pharynx, being for a time fused with it. It then re-enters the cranial base and runs rostrally to end just caudal to the hypophysis (**3.137A**).

During the fifth week bilateral *otocysts* (auditory vesicles) become enclosed within the *otic capsules*, which soon differentiate into dorsolateral *vestibular* and ventromedial *cochlear* parts, enveloping the primordia of the semicircular canals and the cochlea. Between these two regions the facial nerve lies in a deep groove. The otic capsules fuse with the lateral processes of the

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occipital plate, leaving a wide hiatus through which the internal jugular vein and the glossopharyngeal, vagus and accessory nerves pass. At this stage the mesenchyme around the developing hypophyseal stalk, which is forming the rudiment of the postsphenoid part of the sphenoid bone, spreads out laterally to form the future greater wings of this element. Smaller processes rostral to this indicate the sites of the lesser wings of the sphenoid, while other condensations reach the sides of the nasal cavity and also blend with the still mesenchymatous septum.

### Basal Regions of the Skull

These are, in mammals, initially preformed in cartilage (**3.137**). This occurs primarily in three regions:

- caudally, in relation to the notochord
- intermediately, in relation to the hypophysis
- rostrally, between the orbits and the nasal cavities.

These may be named *parachordal*, *hypophyseal* and *interorbitonasal* regions. The *parachordal cartilage* is developed from paraxial mesenchyme related to the cranial end of the notochord and the first five (occipital) somites; caudally it exhibits traces of four primitive segments separated by the roots of the hypoglossal nerves. It is notable that the region of fusion between the rostral part of the occipital bones and the portion of the parachordal plate that is of somitomeric origin corresponds to the spheno-occipital synchondrosis, which is the site of growth for up to 20 years of age. The *otic capsule* is formed from three different sources (identified in the chick): the first somite, a portion of paraxial mesenchyme and neural crest mesenchyme (Couly et al 1992).

The *hypophyseal cartilage* ossifies to form the *postsphenoid part* of the *sphenoid bone*; it derives from both paraxial mesenchyme and neural crest in the chick (Couly 1992) (see also **3.101**). The paraxial mesenchyme contributes to the caudal part of the sella turcica, forming each side of the rostral end of the notochord, whereas the neural crest forms the more rostral portion of the sella turcica, and the region termed by Couly et al (1992) the *prechordal skeleton*. The interorbitonasal cartilage is perhaps to be equated with the trabeculae cranii of lower vertebrates and is usually known as the trabecular cartilage, which is a bilateral structure developing from two centres of chondrification. The *trabeculae cranii* and the *ethmoid* complex are of neural crest origin.

In the human embryo cranial chondrification begins in the second month; cartilaginous foci first appear in the occipital plate, one on each side of the notochord (parachordal cartilages); these later fuse at the end of the seventh week surrounding the notochord, whose oblique transit through the region has been mentioned (**3.137A**). The cartilaginous posterior part of the sphenoid is formed from two hypophyseal centres, flanking the stalk of the hypophysis and uniting at first behind, then in front, enclosing a *craniopharyngeal canal* containing the hypophyseal diverticulum. The canal is usually obliterated by the third month; its association

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with the derivation of the anterior lobe of the hypophysis from the pharyngeal diverticulum of Rathke has been denied.

The otic capsules, presphenoid, bases of the greater wings and lesser wings of the sphenoid, and finally the nasal capsules, in turn become chondrified. The whole nasal capsule is well developed by the end of the third month, consisting of a common median septal part (sometimes initially termed the *interorbitonasal septum*) and two lateral regions. The free caudal borders of the latter incurve to form the interior nasal conchae, which ossify during the fifth month and become separate elements. Posteriorly each lateral part of the nasal capsule becomes ossified as the ethmoidal labyrinth, bearing on its medial surface ridges the future middle and superior conchae. Part of the rest of the capsule remains cartilaginous as the septal and alar cartilages of the nose; part is replaced by the mesenchymatous vomer and nasal bones.

The ventral surface of the chondrocranium is associated with the cartilages of the pharyngeal arches, the development of which will be considered later (see p. 275 et seq). The bones of the cranial base which are thus preformed in cartilage are the occipital (excepting the upper part of its squama), the petromastoid part of the temporal, the body, lesser wings and roots of the greater wings of the sphenoid, and the ethmoid. These constitute the cartilaginous part of the neurocranium. To summarize, therefore, the base of the skull—except for the orbital plates of the frontal and the lateral parts of the greater sphenoidal wings—is preformed in cartilage (**3.137**, see also **3.101**).

Specification of the pattern of the base of the skull may be caused by an epithelial/mesenchymal interaction involving the overlying neural tube. Thorogood (1988) proposed a 'flypaper model' of development for the cartilaginous neurocranium. The basal aspect of the neuroectoderm transiently expresses type II collagen around the olfactory regions, around the optic cups prior to and during invagination of the lens, around the otic vesicles, and on the ventrolateral surfaces of the diencephalon, mesencephalon and rhombencephalon (Thorogood 1988). The notochord also expresses type II collagen in its perichordal sheath. Some time later, after the neural expression of type II collagen has ceased, mesenchyme adjacent to the regions described above commences synthesis of type II collagen and begins differentiation into chondrocytes. Thorogood reasoned that the transient expression of type II collagen in the basal lamina of the neural epithelium causes localized arrest of cell migration of those mesenchyme cells which touch it, regardless of origin. (A similar mechanism is seen in the notochordal–sclerotome interaction; see p. 265.) At such sites cells accumulate and undergo a matrix-mediated interaction with the neural epithelium and differentiate along a chondrogenic lineage. Thus the pattern in which the cells are trapped, epigenetically determines the form of the chondrocranium. This has evolutionary implications, as slight alterations in the expression of the type II collagen by the neuroepithelium could have profound effects on the shape and form of the chondrocranium and on the whole skull, because the blastemal skull must connect to the plan initiated by the chondrocranium. This model could perhaps account for the diversity of skull shape seen in the vertebrates.

Ossification commences before the chondrocranium has fully developed, and as this change extends, bone overtakes cartilage until little of the chondrocranium remains. However, parts of it still exist at birth and small regions remain cartilaginous in the adult skull. At birth unossified

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chondrocranium still persists at:

- the alae, lateral nasal and septum of the nose
- the spheno-ethmoidal junction (p. 551)
- the spheno-occipital and sphenopetrous junctions (p. 551)
- the apex of the petrous bone (foramen lacerum)
- and also between ossifying elements of the sphenoid bone and between elements of the occipital bone.

Most of these regions function as growth cartilages. For further development of these areas and cranial bones in general see Section 6, Skeletal system.

### *Vault or Upper Regions of the Skull*

These first appear about the thirtieth day; they consist of curved plates of mesenchyme at the sides of the skull and gradually extend cranially to blend with each other; they also extend towards and reach the base of the skull, which will become part of the chondrocranium. The mesenchymatous (membranous) neurocranium, corresponding to the cranial vault, is not preformed in cartilage. Its elements, frequently described as dermal bones (p. 548), are the frontal bones, the parietals, the squamous parts of the temporal bones and the upper (interparietal) part of the occipital squama. It is now believed that the frontal, parietal and squamosal bones are formed from neural crest. Also the sutures of the calvarium and facial bones are made up of crest cells (Couly et al 1992).

There is a close association between the developing meninges, particularly the dura mater, and the calvarial bones. The dermal bones are formed by the initiation of a wave of osteodifferentiation moving radially from ossification centres within the desmocranial mesenchyme. When adjacent bones meet, proliferation of the osteogenic front ceases and sutures are induced to form. Once sutures are formed and the fibrous desmocranium is replaced by mineralized bone, a second phase of development occurs in which growth of the cranial bones occurs at the sutural margins (Opperman et al 1993). Such growth forms most of the calvaria.

Opperman et al (1993) have demonstrated that transplants of sutures in which the fetal dura mater is left intact results in a continuous fibrous suture between developing vault bones, whereas in transplants in which the fetal dura mater is removed bony fusion occurs. This interaction of underlying dura mater with the developing calvarial bones has been demonstrated experimentally, showing that the dura not only promotes the position and maintenance of sutures, but also that dura can repattern both the reappearance and position of the bones and sutures of the cranial vault after removal of the calvaria in the neonate.

At the site of a developing suture the osteogenic fronts of two adjacent bones meet and overlap. Initially there is a highly cellular suture blastema between the bones which later becomes more

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dense and acellular. In the mature suture a narrow overlap of compact bone contains a dense, narrow band of cells continuous with the periosteum.

### *Musculature Associated with the Neurocranium*

Most of the striated musculature of the head is formed during development of the viscerocranium when muscle masses, particularly from the second pharyngeal arch, migrate to cover parts of the neurocranium (see p. 284). However, two further sources of muscle provide myoblasts for the external ocular muscles and the tongue.

## Extrinsic Ocular Muscles

All extrinsic ocular muscles derive from prechordal mesenchyme which lies at the rostral tip of the notochordal process and remains mesenchymal after the notochordal process becomes epithelial and gains a basal lamina (**3.148**). In early embryos prechordal mesenchyme migrates laterally towards the paraxial mesenchyme (p. 144). Its early myogenic properties in the head can be demonstrated by chimeric recombination, and further, if transplanted into limb buds it is able to develop into muscle tissue.

Early embryos develop bilateral cavities in the head, previously described as preotic somites. The walls of the premandibular head cavities are lined by flat or cylindrical cells which do not exhibit the characteristics of a germinal epithelium like the epithelial plate of the somite; also there is no basal lamina around the head cavities. As the oculomotor nerve grows down to the level of the head cavity a condensation of premuscle cells appears at the ventrolateral side of the head cavity. Later the head cavities are filled with ingrowing mesenchyme. The premuscular mass subdivides into the blastemata of the different muscles supplied by the oculomotor nerve (Wachtler & Jacob 1986). Similar events occur with respect to the intermediate head cavity (trochlear nerve and superior oblique muscle), and the caudal head cavity (abducent nerve and lateral rectus muscle).

There is no doubt that the head cavities are formed by a mesenchymal/epithelial shift similar to that seen in the somites. However, the epithelial plate of the somite is a germinal centre which produces postmitotic myoblasts destined for epaxial regions, and migratory premitotic myoblasts destined for the limbs and body wall. The head cavities may serve a similar purpose if a mesenchyme/epithelial shift is part of a maturation process for putative myoblasts; however, it may not need to provide a centre for cell replication: premitotic myoblasts differentiated directly from the prechordal mesenchyme may form the premuscular masses.

## Muscles of the Tongue

This development appears to be similar to the development of the muscles of the limb. Single, premitotic cells detach from the ventrolateral portion of the occipital somites and migrate to their ultimate positions (Wachtler & Jacob 1986) (**3.131**, **148**). The connective tissue

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surrounding these muscles is derived from neural crest cells (Noden 1983).

## Viscerocranium

The development of the viscerocranium is very complex. It involves the migration and interaction of epithelial populations derived from: the neural folds, surface ectoderm and endoderm; mesenchymal populations from the mesencephalic, metencephalic and myelencephalic neural crest, paraxial mesenchyme and angiogenic mesenchyme; and neural populations from the neural tube, neural crest and ectodermal placodes. Generally, the more rostral structures, i.e. face, palate, buccal cavity and nasal cavity, derive entirely from ectodermal populations (both epithelium and mesenchyme—via neural crest), whereas the caudal and related structures, i.e. pharynx and larynx, are derived from ectoderm, neural crest and interactions with endoderm.

The development of the face and neck is intimately related to the development of the brain and special sense organs; the reader is advised to refer to the development of the neural crest on pages 147 and 220, and of the head, page 157, and to **3.100**, **101**, **148**.

All of the structures which give rise to the face and neck are segmentally organized during development; the hindbrain displays rhombomeres, the ectoderm—ectomeres and the paraxial mesenchyme—somitomeres. The overall segmentation of this region is related to the expression of axial genes in the head which have been conserved throughout evolution.

### *Vertebral Pharyngeal Apparatus*

In all vertebrate embryos, after head fold formation the *stomodeum*, or primitive mouth, is bounded cranially by the projecting forebrain and caudally by the cardiac prominence (**3.138** ). The mandibular region and the whole of the neck, which will subsequently intervene between mouth and developing thorax, are absent, but will be formed by the appearance and modification of six paired *branchial* (*aill*) *arches*, which develop in the lateral aspects of the head adjacent to the hindbrain (**3.139**, **140**, **141**). In the earliest vertebrates which were jawless (*Agnathia*), the branchial arches were a uniform series of bars behind the gill clefts; but long before the evolution of the terrestrial vertebrates, remarkable adaptations had occurred in them. Structures commonly regarded as the first pair of arches became the jaws, upper and lower, of the jaw-bearing vertebrates (*Gnathostomata*), including most fish; they are, therefore, usually named the *mandibular arches*. (The term 'mandibular arch' is widely used but not entirely appropriate because of the numerous maxillofacial, nasal, otic and palatopharyngeal derivatives from its dorsal end.) It should, however, be noted that since this early identification, strong evidence has accumulated that, at least, a pair of *premandibular arches* existed and have become adapted as the *trabeculae cranii* of subsequent vertebrate embryos. These are probably represented by the interorbitonasal cartilage of the human embryo (see p. 271) which forms a branchial element in the chondrocranium. The next (*postmandibular*) arch in the series is the *hyoid arch*; its skeletal derivatives form the varied hyoid elements present in all vertebrates with

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jaws. The most dorsal of the latter, the *hyomandibula*, is already present in cartilaginous fish as a strut between the skull and the primitive jaw joint, thereby reducing the cleft between the mandibular and hyoid arches to a small opening, the *spiracle*. The interesting further evolution of this region in land animals in connection with the auditory apparatus has been considered (p. 263). The hyoid arch also contributes to the formation of a gill cover, or *operculum*, in bony fish, and the remaining arches persist as the supports of the gill apparatus.

At first the arches produce rounded ridge-like prominences both of the overlying ectoderm and of the endodermal lining of the lateral walls and floor of the pharynx. In the furrows between these prominences the ectoderm and endoderm are in virtual contact. The thin membranes so formed break down permanently in gill-breathing vertebrates, transiently in reptile embryos, but persist in the tetrapods, in which open channels or 'true clefts' are not formed. However, the external *branchial grooves* which correspond to them are frequently, less appropriately, called *branchial clefts* and their internal counterparts are the *pharyngeal sacs* or *pouches*.

In gill-breathing vertebrates the exchange of respiratory gases is directly from solution in water to solution in blood. From the cranial end (arterial, but carrying deoxygenated blood) of the heart emerge two *ventral aortae* which traverse the ventral pharyngeal wall, sending branches curving dorsally into the branchial arches, where they feed capillary plexuses in the gills. These are drained by corresponding arteries, which join two *dorsal aortae* supplying the general circulation. As water is taken in through the mouth and passed back through the gill clefts, its dissolved oxygen diffuses through the pharyngeal endoderm and endothelium of the gills to reach the blood, carbon dioxide diffusing out of the latter into the water. This intimate relationship between the developing mouth, branchial apparatus and heart in water-breathing vertebrates is repeated in the embryos of their tetrapod descendants, but with many modifications necessary to changed respiratory function.

Although a description of the branchial apparatus is appropriate for water-breathing vertebrates, the application of this terminology to mammalian embryos is by no means universal. O'Rahilly and Muller (1992) consider the term *branchial* to be inappropriate for mammalian embryos. Similarly the term *visceral*, used synonymously to describe the arches, has been questioned by Noden (1991) who notes that 'visceral' suggests a primary relation between the arches and the internal pharyngeal tube that obscures the somatic function of most of the tissue within the arches. The region of the embryo containing the rostral foregut, surrounded by mesenchyme and ectoderm, constitutes the embryonic pharynx; the stage of development at which the arches are prominent has been termed the *pharyngula stage (*see p. 100). However, the appellation *pharyngeal* arches is also problematical as the first arch which forms most of the face is in the main composed of ectoderm alone, both on the outer and inner surfaces and within the arch (neural crest mesenchyme). Thus the first arch is technically not a pharyngeal structure, unlike the subsequent caudal arches which are composed of ectoderm externally, endoderm of the pharynx internally and neural crest mesenchyme within the arches. This difference in origin of the first and subsequent arches is related to the evolution of the head and skull (see p. 287). For the purposes of the description of human embryology the term *pharyngeal* arches will be used; however, comparison with other species will involve the term branchial.

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### *A Typical Pharyngeal Arch*

Generally each pharyngeal arch consists of an epithelial covering exteriorly and a mesenchymal core interiorly (**3.139**, **140**, **141**). The epithelium may be ectodermal entirely (as in the first arch), or ectoderm covering the external aspect of the arch and endoderm covering the internal aspect of the arch (as in the remaining arches). The mesenchyme within each arch derives from neural crest, paraxial and angiogenic mesenchymal populations. The motor and sensory roots of a cranial nerve are associated with the epithelium and mesenchyme of each arch.

From these disparate cell populations each arch develops:

- region-specific *epithelial structures*
- a *skeletal element* from the neural crest mesenchyme
- associated *striated muscle* from the paraxial mesenchyme
- an *arch artery* from the angiogenic mesenchyme
- *motor* and *sensory nerves* specific to the arch.

*The epithelia* covering each arch is patterned by the underlying mesenchyme. Such patterning is specific for individual arches and results in such diverse specializations as: keratinized stratified squamous epithelium, hair, sweat, sebaceous and ceruminous glands; pseudostratified ciliated columnar epithelium, teeth, salivary, mucous and lacrimal glands; the epithelia of glands such as the thyroid, parathyroids, thymus; and of the lymphoid tissues in the oro- and nasopharynx.

*The skeletal element* is formed from neural crest mesenchyme which condenses and subsequently chondrifies either wholly or in part of its length; if this change is complete the element extends dorsally until it comes into contact with the mesenchymatous cranial base lateral to the hindbrain. The arch cartilage, entirely or in part, may remain as cartilage, undergo endochondral ossification, be replaced completely by intramembranous ossification, or become ligamentous. Neural crest also gives rise to the *ligaments*, *tendons* and *connective tissue* in the arches and the *dermis* underlying the skin. Generally the neural crest controls the pattern of development of the arches and is itself programmed by the expression of *Hox* genes in the hindbrain (see p. 227).

*The striated muscle of each arch*, sometimes termed *branchial musculature* to denote its origin, derives from the unsegmented paraxial mesenchyme of the head, the somitomeres (see pp. 154, 285); the myoblasts may migrate great distances and lose connection with the skeletal elements in the arches which cease their original respiratory function. The identities of these muscle masses, where they assume new functions, can nevertheless be inferred by reference to their nerve supply.

*An arch artery* develops in each arch either by vasculogenesis, where angioblastic mesenchyme

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migrates into a region and initiates vessel development in situ, or by angiogenesis, where vessels develop by sprouting from the endothelium of pre-existing vessels (see p. 299). The paired arch arteries arise from the truncus arteriosus and pass laterally each side of the pharynx to join the dorsal aortae.

*Nerves* arise from the adjacent hindbrain (**3.96**, **112**). They pass directly into the arches, which are ventral to it, by two methods. *Motor nerves* grow out from the basal plate of the midbrain and hindbrain to innervate the striated muscle in the arches. Generally these nerves are termed *special branchial efferent* noting their innervation of branchial musculature. *Sensory nerves* extend from cranial sensory ganglia which are derived in part from neural crest cells and in part from ectodermal placodes (see p. 237); they convey *general* and *special somatic afferent* axons. Within the arch a mixed nerve typically runs along the rostral border and is hence described as *post-trematic*, because it is behind or caudal to the cleft or trema rostral to the arch. A sensory branch from this principal post-trematic nerve passes to the immediately rostral arch where it runs close to the caudal border; it is thus *pretrematic* with respect to the cleft caudal to it (**3.139**). In the human embryo the pre- and post-trematic nerves cannot be identified with certainty.

## Development of the Pharyngeal Arches

The human circumoral *first pharyngeal arch* (**3.138**, **139**) consists, on each side, of two main regions: a *ventral part* or *mandibular prominence* and a *dorsal part* or *maxillary prominence*. Each mandibular prominence, first seen at stage 10 (22 postovulatory days), grows ventromedially in the floor of the pharynx to meet its fellow in the midline, being situated between the primitive mouth and the cardiac (pericardial) prominence. The maxillary prominences are not seen until stage 13; their enlargement coincides with proliferation of neural crest mesenchyme between the ectoderm and prosencephalon forming the frontonasal prominence (see below). The enlargement of the first arch is particularly rostral to the site of the buccopharyngeal membrane; thus inner and outer aspects of this arch are covered with ectoderm. The *second* or *hyoid arches*, seen from stage 11, are caudal to the maxillomandibular; they similarly grow ventrally to meet and fuse in the midline. The *third arches* are seen at stage 12 (26 days) and the *fourth arches* by stage 13 (28 days); the latter especially are not prominent, being largely sunk in a depression produced by the caudal overlapping of the hyoid arch. The *fifth* and *sixth arches* cannot be recognized externally and can only be identified by the arrangement of the mesenchyme and by slight projections into the pharynx.

## The First Pharyngeal Arch

The first pharyngeal arch is sufficiently different, both in its structure and development, from the subsequent caudal arches for its separate examination. Unlike the other arches it possesses dorsal and ventral prominences, appearing C-shaped in lateral view (see **3.142**). The dorsal (maxillary) prominences interact with ectodermal epithelia and neural crest mesenchyme of the frontonasal prominence, and generally form more extensive skeletal structures than the other

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arches (see p. 284); particularly, these skeletal elements fuse with the chondrocranium. The first arch is completely clothed with ectoderm unlike the caudal arches which are dependent on the proximity of pharyngeal endoderm for their development. The ectoderm originates (in the 3-somite chick) from a territory lateral to the mesencephalic neural folds (see **3.145**). The mesencephalic folds themselves give rise to both the ectodermal placodal cells and neural crest cells which contribute to the trigeminal ganglion, and the mesenchymal population which streams into the mandibular and maxillary prominences.

The first arch contains on each side a dorsal and ventral cartilage. The former represents the *palatopterygoquadrate bar*, a prominent element in earlier vertebrates forming part of the upper jaw but much reduced in mammals. In human embryos its early appearance seems transient and its contribution to some permanent cranial structures, such as the maxilla, is uncertain (however, see below). The *ventral cartilage* (of Meckel, **3.143**) extends from the developing otic capsule into the mandibular prominence, meeting its fellow at its ventral end. The dorsal end of Meckel's cartilage becomes separated, and was often held to form the rudiments of both *malleus* and *incus*. However, there is strong palaeontological (Romer 1970) and comparative anatomical (Shute 1956) evidence that the incus is, in part, to be regarded as a homologue of the *quadrate bone* of reptiles, and it is therefore probably more correctly regarded as a derivative of the palatopterygoquadrate cartilage. This cartilage may also contribute to the ala major of the sphenoid bone and the roots of its pterygoid plates. Beyond the rudiment of the malleus, the intermediate part of Meckel's cartilage disappears, but its sheath persists as the *anterior malleolar* and *sphenomandibular ligaments*. The ventral part, much the largest, is enveloped by the developing mesenchymatous mandible (p. 577); a small fraction of this, extending from the mental foramen almost to the site of the future symphysis, probably becomes ossified from invading mandibular tissue, into which it is incorporated, while the remainder of the cartilage is ultimately absorbed.

The cells which give rise to the muscle of the first arch arise from the paraxial mesenchyme localized to somitomeres 2 and 3 (Trainor et al 1994) (p. 285). The muscle mass of the mandibular part of the first arch forms the *tensor tympani*, *tensor veli palatini* and the *masticatory muscles*, including *mylohyoid* and the *anterior belly of digastric* (**3.144**). The tensor tympani retains its connection with the skeletal element of the arch through its attachments to the malleus, and the tensor veli palatini to the base of the medial pterygoid process, which may be derived from the dorsal cartilage of the first arch, but the masticatory muscles transfer to the mandible, a dermal bone.

All these muscles are supplied by the mandibular nerve, the mixed 'post-trematic' nerve of the first arch.

### Face

While the mandibular prominences are invading the floor of the pharynx, mesencephalic neural crest cells migrate rostrally and laterally between the prosencephalic neuroepithelium and the surface ectoderm to form the extensive *frontonasal prominence*. During the fifth week the sites

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of the *olfactory* or *nasal placodes* are established ventrolateral to the frontonasal prominence, dividing the latter, on each side, into *medial* and *lateral nasal prominences* or folds; the olfactory placodes originate from the neural folds (p. 222). The placodes are at first widely separated and coplanar with the surface ectoderm but, as the nasal prominences develop, they soon become depressed to form the *olfactory pits* (nasal sacs). The olfactory placodes are the anlage of the olfactory and vomeronasal epithelia, which derive from the rostral neural folds; these folds also give rise to the respiratory epithelia of the nasal cavity (see **3.145**). The lateral nasal prominences are the more evident (**3.142**, **3.146B**), but the medial nasal prominences, still separated by the median remainder of the frontonasal field, project caudally beyond the former. Extensions of mesenchyme from the medial prominence into the roof of the stomodeum proliferate to form the *premaxillary* fields. Each nasal sac has a ventral fold from which develops an epithelial *nasal fin* passing caudally to fuse with the stomodeal roof.

While these changes are progressing a somewhat triangular elevation swells ventrally from the cranial aspect of the dorsal region of each mandibular prominence. This is the *maxillary prominence*, and like the frontonasal prominence it consists of proliferating neural crest mesenchyme covered by ectoderm. Each maxillary prominence grows in a ventral direction and fuses with the lateral nasal prominence, the two being at first separated by a *nasomaxillary groove* (*naso-optic furrow*) (**3.142**; Streeter 1948). The opposed margins of the lateral nasal and maxillary prominences growing together thus establish continuity between the side of the future nose and the cheek (**3.142**). The ectoderm along the boundary between them does not entirely disappear; it gives rise to a solid cellular rod, which at first develops as a linear surface elevation, the *nasolacrimal ridge*, and then sinks into the mesenchyme (Politzer 1952). Its caudal end proliferates to connect with the caudal part of the lateral nasal wall, while its cranial extremity later connects with the developing conjunctival sac. The solid rod becomes canalized to form the *nasolacrimal duct* (**3.146B**).

(It should be noted that the epithelial folds and elevations due to loci of proliferation of underlying mesenchyme were long termed processes. The International Nomenclature Committee felt that this was not entirely appropriate and their revised term 'prominence' has been adopted here. Both terms are used in the literature.)

The relatively wide primitive mouth or *stomodeal fissure* is progressively reduced, and the epithelial and connective tissues of the cheek enlarged, by fusion between the adjacent surfaces of the mandibular and maxillary prominences. This proceeds from the para-otic region to the angle of the definitive *oral fissure*.

### Nasal Cavity

The rounded apex of the triangular maxillary prominence extends beyond the lateral nasal prominence, crossing the caudal end of the olfactory pit to meet and fuse with the *premaxillary elevation* developing at the extremity of the frontonasal field. This closes off the lower or caudal edge of the olfactory pit, the upper part of the opening of which is thus defined as the primitive *external naris*. The growth of the surrounding mesenchyme leads to a deepening of the pit to

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become a primitive nasal cavity, or *nasal sac*, the epithelial wall of which, in the dorsocaudal part of its extent, the nasal fin, retains contiguity with the epithelium of the stomodeal roof. This contact area becomes progressively greater as growth continues, and the nasal fin is eroded, ultimately forming a thin layer, the *oronasal membrane* (**3.146A**), which also disappears later. Thereafter, the primitive nasal cavity communicates with the stomodeum through a primitive *internal naris* (*choana*), which is at this stage still well forward or ventrally situated in the stomodeal roof (Warbrick 1960). By these changes a new cranial boundary is set for the oral opening, consisting of the fused premaxillary and maxillary regions. This is the future upper lip, but it has not yet become separated from the deeper tissues which will form the maxillary alveolus. At the same time the nasal cavity acquires a floor through the fusion of the nasal prominences and the maxillary prominences. At this stage the two external nares are still widely separated by an area derived from the frontonasal field, but this separation becomes reduced by the fusion of the premaxillary mesenchyme from the two sides. According to some investigators the mesenchyme of the maxillary prominences invades the premaxillary regions, the mesenchyme of which is said to become buried, to form later the premaxilla or os incisivum (p. 574; Boyd 1933; Baxter 1953). The maxillary mesenchyme is thus considered by some to contribute substantially to the formation of the *philtrum* of the upper lip, thus accounting for its maxillary innervation. Others, however, maintain that the philtrum is derived wholly from premaxillary tissue (Keith 1948; King 1954; Warbrick 1960; Wood et al 1967) (see also **3.100**). The maxillary nerve primarily innervates the maxillary mesenchyme but apparently extends later into the territory of the frontonasal prominence. It should be added that some workers deny that sensory nerve distribution is a reliable guide to migration of mesenchyme in the case of the maxillary prominence.

### *Palate*

Once the primitive nasal cavities are defined the ventral part of the roof of the oral cavity can be regarded as the *primitive palate* (*median palatine prominence*; **3.146A**). It is formed by the premaxillary regions and maxillary prominences, which become confluent and establish continuity with the thick median *nasal septal prominence* (*primitive nasal septum*). As the head grows in size, the region of mesenchyme between the forebrain and oral cavity increases greatly by proliferation and the nasal cavities deepen, extending towards the forebrain. Simultaneously they also extend dorsally from the primitive choanae as two narrow and deep grooves in the oral roof (**3.146**) which are separated by a partition. The grooves and the partition deepen together, and the latter becomes the *nasal septum*, continuous rostrally with the *primitive nasal septum* (**3.146B**). The broad dorsocaudal border of the nasal septum is at first in contact with the dorsum of the developing tongue (**3.146B**), the right and left nasal cavities still communicating freely with the mouth except where the nasal floor is already established ventrally by the primitive palate.

During Stage 17 (41 days) the internal aspects of the maxillary prominences produce *palatine processes* (*shelves*), which grow towards the midline but are for some time separated from each other by the tongue. At this stage the roof of the oral cavity projects ventrally beyond its floor and the tip of the developing tongue actually lies in contact with the cranial (superior) surface of the primitive palate. A coronal section dorsal to this shows the maxillary palatine processes

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contiguous with the sides of the tongue and bent into a vertical position on each side of it (**3.146B**). With further growth, the mandibular region and the tongue are carried forwards (ventrally), and the lingual tip passes round to the caudal surface of the primitive palate. At stage 23 (56–57 days) the palatine processes rapidly elevate, assuming a horizontal position which allows them to grow towards each other and thus to fuse (**3.146C**); this occurs from before backwards.

The change of position occurs very rapidly caused by the progressive region specific synthesis and accumulation of hyaluronic acid within the palatal process mesenchyme. The hyaluronic acid will bind up to 10 times its own weight of water, thus causing swelling and expansion of the palatial shelves. This process is further aided by the alignment of collagen fibrils and palatal mesenchymal cells (the latter contract in response to acetylcholine and serotonin which they secrete thus regulating the elevation of the shelves), and by the epithelium which restrains the swelling. Once these forces are in concert and exceed the resistance factors, the palatal shelves will mechanically elevate. Such elevation occurs at a time of craniofacial growth when there is constant growth in head height but almost no growth in head width. This latter factor is important: if palatal shelf elevation is delayed so that they elevate in a period of growth in facial width, the unfused processes are unable to touch physically and cleft palate may result. Other factors affecting palatal closure are the growth in length of the first arch cartilage (Meckel's) which allows the tongue to lower into the developing mandible. Further, the change in position of the maxilla relative to the anterior cranial base, which is maintained at about 84° during weeks 9 and 10, has the effect of lifting the head and upper jaw upwards from the mandible so permitting withdrawal of the tongue from between the palatal shelves and creating space for them to elevate. Mouth opening, tongue protrusion and hiccup movements have also been noted at this time; these movements and their associated pressure changes may assist palatal shelf elevation (Ferguson 1977, 1990, 1993). Generally in female embryos palatal shelf elevation occurs 7 days later than in males, making congenital cleft palate more likely in female embryos. After elevation the palatine processes grow medially along the inferior borders of the primitive choanae, uniting with them and with the margins of the median palatine prominence, except over a small area in the midline where a *nasopalatine canal* maintains connection between the nasal and oral cavities for some time and marks the future position of the incisive fossa. (The plates which form the early (primitive) palate are sometimes known as *median* palatine processes, the maxillary contributions being then named the *lateral* palatine processes.)

As the medial borders of the maxillary palatine processes fuse together, fusing also with the free border of the nasal septum, the nasal and oral cavities are progressively separated and the tongue is excluded from the former. The nasal cavities are thus extended dorsally and the choanae reach their final position, leaving the caudal edge of the nasal septum free in about its dorsal quarter as the partition between them. Slightly later the dorsomedial extremities of the palatine processes, which extend dorsally beyond the choanae, fuse together rostrocaudally to form the future epithelia and connective tissues of the soft palate (**3.146C**). There is later an upgrowth of myogenic mesenchyme from the third and, probably, other pharyngeal arches into the palate and around the caudal margins of the auditory tube, along a line corresponding in the final state to the palatopharyngeal arches (Baxter 1953).

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On each side of the nasal septum, in a ventral or anterior position just above the primitive palate, placodal ectoderm is invaginated to form a pair of small diverticula, which extend dorsally and cranially into the septum. These are vestiges of the *vomeronasal organ* (**3.146C**), whose openings are close to the junction between the two premaxillae and the maxillae; they are always rudimentary in mankind, but are well-developed auxiliary olfactory organs in many vertebrates (pp. 1225, 1321). For bibliographies in the field of facial development consult Latham (1973).

### *Facial Epithelium*

The external ectoderm over the mandibular prominences becomes the skin of the face (**3.147**), and it also takes part in forming the tragus of the auricle (p. 1368). Its surface facial contribution is roughly triangular; the apex includes the tragus, the upper border extending to the lateral angle of the mouth and free border of lower lip; its lower border curves to follow the principal submandibular flexure line of the neck. The surface facial contribution of the maxillary prominence extends from the supratragic point to the lateral angles of eye and mouth, includes the lower eyelid and follows the paranasal line of the nasolacrimal duct, finally including a controversial amount of the upper lip.

The ectoderm on the arched, circumoral borders of both the mandibular and maxillary prominences, including the premaxilla medially, thickens along two curved parallel arches. The external thickening is the *labiogingival* or *vestibular lamina*, and the internal the *dental lamina*. The labiogingival lamina invades the subjacent mesenchyme and subsequently breaks down to form a sulcus (the vestibule) which separates the lower and upper lips from their adjacent gums. Within the mandibular prominence, the gum is separated from the tongue by the *linguogingival groove.* The dental lamina denote the sites of development of the enamel organs of the teeth.

## Teeth

Teeth form from a series of epithelial/mesenchymal interactions along the dental lamina. In 27-mm embryos individual dental laminae expand into little ectodermal (dental) sacs surrounded by vascular mesenchyme. The ectoderm proliferates to form an *enamel organ* which surrounds a local portion of neural crest mesenchyme, the *dental papilla*; together this unit is a *tooth bud* or *germ*. The enamel organ initially forms a cap over the dental papilla then later it expands into a bell shape, the inner layer tightly adherent to the dental papilla and separated from the outer layer by accumulated glycosaminoglycans (GAGs). The inner cells of the enamel organ differentiate into *ameloblasts*, and the underlying mesenchymal cells into *odontoblasts.* Tooth development is further considered on page 111; the interactions associated with tooth development are considered below.

Both the deciduous and permanent teeth are formed as above. The permanent teeth develop in accessional positions from the lingual aspects of the existing tooth germ; however, the tooth germs for the 12 permanent molar teeth develop from posterior extensions of the dental laminae on each side of both jaws. Calcification begins in both deciduous and permanent teeth before

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birth; the deciduous teeth have well-developed crowns by full term, whereas the permanent teeth remain as tooth buds.

Just as the neural crest mesenchyme is responsible for the patterning of the pharyngeal arches, so it directs the pattern of tooth development. Thus, dental papilla mesenchyme is able to induce the formation of teeth in non-oral epithelium, and can specify the type of tooth produced, i.e. incisor or molar. Reciprocal interaction between the cells of the tooth germ in response to the extracellular matrix they secrete occurs, i.e. secretion of predentin by odontoblasts stimulates the differentiation of the inner enamel organ into ameloblasts which secrete enamel.

When cranial neural crest is cultured alone it will differentiate into cartilage. If it is recombined with limb epithelium then cartilage and bone will form. However, when cranial neural crest is recombined with mandibular epithelium, salivary islands, hair and teeth form as well as cartilage and bone. Thus the mandibular epithelium is essential and specific for the development of teeth. At a local level, early (9–11.5 days) recombination of mouse mandibular epithelium and second arch mesenchyme results in teeth in 90% of cases, whereas the reverse recombination, second arch epithelium and mandibular arch mesenchyme, does not produce teeth. Later recombination experiments (11.5–12 days), where first arch mesenchyme is grafted with second arch epithelium, will produce teeth, leading to the conclusion that the crest mesenchyme becomes specified to produce teeth after day 12 (Kollar & Mina 1991). This specification can be changed experimentally. If presumptive incisor region of the mandibular epithelium is recombined with predetermined molar papillae from post-day 12 tooth germs, the shape of the teeth can be redefined by the epithelium and incisiform teeth develop.

The mesenchymal dental papilla can influence epithelia from different species; thus recombination of dental mesenchyme from 16–18-day mouse with oral epithelium from the mandibular epithelium of the chick resulted in tooth formation (Kollar & Fisher 1980). This is the more surprising as chicks do not normally develop teeth. Similarly recombination of chick arch mesenchyme and 10-day mandibular epithelium from the mouse resulted in tooth formation (Kollar & Mina 1991). In the latter case the epithelium initiated the dental papilla development.

## *Anomalies of Facial Development*

Congenital malformations consequent upon arrest of development and failure of fusion of components in the formation of the face and palate are not uncommon. At the simplest, one maxillary prominence may fail completely to fuse with the corresponding premaxillary region (globular prominence), leading to a persistent fissure between the philtrum and lateral part of the upper lip on that side, *cleft lip* (less appropriately 'hare' lip). A similar but rare malformation follows failure of fusion between the maxillary prominence and the lateral nasal prominence, facial cleft, in which the nasolacrimal duct persists as an open furrow, a condition usually associated with cleft lip on the same side. The palatine processes may fail to fuse with each other and the nasal septum to variable degrees. In its severest form fusion is wholly lacking, leaving a wide fissure between the palatine processes through which the nasal septum is visible. On each side the premaxillary parts of the palate are separated from the maxillary palatine processes by

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clefts which are continuous ventrally with bilateral clefts in the upper lip. In such cases the philtrum is a separate entity, continuous cranially and dorsally with the nasal septum. The floor of the nasal cavity is deficient throughout its extent and the choanae are not completed. Many varieties of milder degrees of cleft palate have been observed; the commonest type is unilateral, only one side of the nasal cavity being in communication with the mouth and the extent of the cleft being variable. In the mildest forms only the soft palate is cleft, or even merely the uvula. Such examples of arrested development may be associated with disturbances in embryonic nutrition during the second and the third months of gestation and the grosser varieties are usually coupled with malformations in other regions of the body (p. 333). In such cases the premaxillary region protrudes, with associated extension forwards of the nasal septum. For discussion see Latham (1973). Certain midline anomalies are rarely encountered, i.e. *median cleft lip* (true hare lip), *cleft nose* and *cleft lower jaw*. More common are minor degrees of *cleft chin* and *micrognathia*—underdevelopment of the lower jaw.

The further growth of the face during the fetal period has received little attention, although this period is by no means characterized entirely by incremental growth. It is during fetal life that human facial proportions develop (p. 371, Fig. **4.28**). The facial and cranial parts display different patterns of growth, though each influences the other. For an interesting analysis of the data observed from 280 fetuses consult Lavelle (1974).

## Caudal Pharyngeal Arches

### *Second Pharyngeal Arch*

The ectoderm covering the outer aspect of the second pharyngeal arch originates from a strip of ectoderm lateral to the metencephalic neural fold (**3.145**), as does the otic placode (these placodal cells are located more laterally than the trigeminal placode in the 3-somite chick embryo). The cartilaginous element of the second arch (Reichert's cartilage) extends from the otic capsule to the midline on each side. Its dorsal end separates and becomes enclosed in the developing tympanic cavity as the *stapes*. Thereafter the cartilage gives rise to the *styloid process*, *stylohyoid ligament*, the *lesser cornu* and probably the *cranial rim* of the body of the *hyoid bone* (**3.143**).

The muscles of the second arch derive from somitomeres 4 and 5. For the most part the muscle mass migrates widely but retains its original nerve supply from the facial. The *stapedius, stylohyoid* and *posterior belly of digastric* remain attached to the hyoid skeleton, but the *facial musculature, platysma, auricular muscles* and *epicranius* all lose connection with it (**3.144**). Their migration is facilitated by the early obliteration of some of the first groove (cleft) and pouch (see below). (This cleft, the spiracle in fishes, is already much reduced in all but the earliest vertebrates.)

### *Third to Sixth Pharyngeal Arches*

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The ectoderm adjacent to the myelencephalic neural fold, down to the level of somite 3, develops to cover the third and fourth pharyngeal arches, a much smaller distribution than that of the more rostral arches. The ectoderm in this region also gives rise to placodal cells which contribute to the petrosal and nodose ganglia. Chondrification does not occur in the dorsal parts of the skeletal elements of the third to sixth arches. The ventral cartilage of the third arch becomes the *greater cornu* of the *hyoid bone* and the *caudal part* of its *body*. (The whole of the body may be formed from the third arch cartilage.) Alternatively, the hyoid body may be derived from cartilage formed in the base of the hypobranchial eminence (p. 175) and thus from third arch tissue alone (Frazer 1926), acquiring its connection with the second arch cartilage secondarily.

The final adaptations of the cartilages of the skeletal elements in the fourth, fifth and sixth arches are a source of disagreement, but the following represents a fairly general view. The *thyroid cartilage* develops from the fourth and fifth arches, which may also give rise to the *arytenoid, corniculate* and *cuneiform cartilages.* The *cricoid cartilage* may be derived from the sixth arch cartilage, or it may be a modified tracheal ring. The *epiglottis* is developed in the substance of the hypobranchial eminence and probably not from 'true' branchial cartilage (**3.144**).

The paraxial mesenchyme from somitomeres 6 and 7 migrates to the third arch and somitomere 7 alone appears to invade the fourth arch (Trainor et al 1994). Somitomeric muscle was not identified in the sixth arch in the mouse. The muscle masses are adapted to form the musculature of the *pharynx*, *larynx* and *soft palate*. The *stylopharyngeus* can be attributed to the third and the *cricothyroid* to the fourth arch (**3.144**). The rest of the laryngeal muscles are derived from the sixth arch and used extensively for vocalization; thus they may not be represented to the same extent in non-human species. The precise origin of the remaining palatal muscles and the pharangeal constrictors is uncertain in man. A mixed origin, partly from paraxial mesenchyme and partly from adjacent myotomes, has been attributed to sternocleidomastoideus and trapezius (McKenzie 1955).

### *Nerves of the Pharyngeal Arches*

The nerves of the pharyngeal arches immediately enter the dorsal ends of them (**3.139**). They are typically mixed, their motor component supplying the muscles of the arch and their sensory fibres innervating the skin and mucous membrane derived from the region. In fish the trunks of the nerves and their ganglia are close to the dorsal ends of the true clefts existing in these forms, each sending a post-trematic branch into its own arch and a pretrematic branch into the arch cranial to this. In mammals, some have claimed that both types of branch can be identified in the first arch, but only a single nerve can be identified with certainty in the second to sixth arches, with the exception of the fifth, the nerve of which is unknown and may have disappeared.

The trigeminal mandibular division is the post-trematic nerve of the first arch; the chorda tympani, or greater petrosal, has sometimes been regarded as its pretrematic nerve derived from the facial. The latter supplies the second arch, the glossopharyngeal the third, the superior laryngeal branch of the vagus the fourth and the latter's recurrent laryngeal branch the sixth. In lower vertebrates the fifth arch is also supplied by a vagal branch. Other branches that have, on

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occasion, been proposed as pretrematic are the tympanic branch of the glossopharyngeal and the auricular branch of the vagus. However, none of the foregoing fulfil sufficient criteria for them to be classified as pretrematic with confidence.

The difference in the courses of the recurrent laryngeal nerves can be explained by the development of the aortic arch arteries. In arches 1–5 the arch nerve enters rostral to its aortic arch artery. However, the nerve enters its sixth arch **caudal** to the aortic arch artery, retaining this position on the left side and hence being caudal to and looping round the ligamentum arteriosum in its final disposition. However, on the right, owing to the disappearance of the dorsal part of the sixth aortic arch artery and the whole of the fifth, the nerve loops round the caudal aspect of the **fourth** aortic arch artery, i.e. the subclavian artery.

### *Muscle of the Pharyngeal Arches*

The muscles of the face and neck (**3.144**), sometimes described as branchiomeric because of their origin within the pharyngeal (branchial) arches, develop from a rostral continuation of the paraxial mesenchyme which, in the trunk, segments to form somites. Within the trunk somites give rise to medial skeletal elements: sclerotomes, which combine to form the vertebrae, lateral myotomic populations; myotomes, which form all of the striated muscle of the trunk and limbs; and limited dorsolateral connective tissue populations which contribute to the dermis over the dorsal surface dermatomes. Experimental quail–chick chimeras have permitted examination of the paraxial mesenchyme in the head to see if similar tripartite fates are available.

Although the paraxial mesenchyme in the head is unsegmented, a segmental pattern was described by Meier (1979) who noted seven *somitomeres* each side of the rostral notochord and beneath the overlying neural plate (see p. 154). Portions of paraxial mesenchyme, medial and lateral to the folding neural plate, were transplanted from quail embryos to chick and the fate of the cell populations followed (Couly et al 1992). At the 3-somite stage the cell density is much higher in the lateral paraxial mesenchyme than in the medial. Apart from the rostral regions of the medial paraxial mesenchyme, which in the avian embryo appeared to contribute to the ocular muscles (these in the main originate from prechordal mesenchyme; see p. 274), the medial mesenchyme gave rise to limited skeletal structures, for example part of the sphenoid and otic capsule, and connective tissues, including the mesencephalic and metencephalic meninges, but no muscles. In contrast, the lateral paraxial mesenchyme developed into the muscular lineages of the pharyngeal arches.

It is interesting that the fate of medial and lateral paraxial mesenchyme corresponds to the medial and lateral fates of the somites. The limited contribution to the dermis seen in the somites has no equivalence in the paraxial mesenchyme. In the head the dermis is formed by neural crest mesenchyme which also has the ability to develop calcified structures.

Prior to the formation of any skeletal elements in the arches, myoblasts migrate from the paraxial mesenchyme to the sites where overt muscle differentiation will occur and form premuscle condensations. The pattern of primary myotube alignment for any one muscle is specified by the

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surrounding neural crest mesenchyme and is not related to the source of the myoblasts. The rate and pattern of muscle maturation are closely associated with the development of the skeletal elements, such that muscles may attain attachments to one skeletal element but remain without additional attachments until the appropriate elements develop (McClearn & Noden 1988). Figure **3.148** shows the relationship between the somitomeres and the muscle masses migrating to each arch.

## Pharyngeal Grooves

Modification of the external contours of the arches occurs as the skeletal and muscular elements develop. The modification of the external *pharyngeal grooves* or (less appropriately) *clefts* produces the smooth contour of the neck. The concurrent development of the internal *pharyngeal pouches* also contributes to this process.

The first pharyngeal groove is obliterated ventrally, as in all but the most ancient vertebrates. In man its dorsal end deepens to form the epithelium of the external acoustic meatus and the external surface of the tympanic membrane. (For details see p. 262.)

At the dorsal ends of the first, second and fourth pharyngeal grooves thickened patches of ectoderm appear, the *epibranchial placodes.* These are closely related to the developing ganglia of the facial, glossopharyngeal and vagus nerves, to which they contribute (p. 224): these, and other placodal cells (*dorsolateral* and *suprabranchial*) also contribute to the trigeminal and vestibulocochlear ganglia (see **3.103**).

At the end of the fifth week the third and fourth arches are sunk in a retrohyoid depression, the *cervical sinus*. Cranially the sinus is bounded by the hyoid arch, dorsally by a ridge produced by ventral extensions from the occipital myotomes and by mesenchyme developing into sternocleidomastoid and trapezius. Caudally, the smaller *epipericardial ridge* separates the sinus from the pericardium and curves cranially near the midline and then with its fellow reaches the lingual swelling of the mandibular prominence and the hypobranchial eminence. Myoblasts from the occipital myotomes migrating to the tongue follow the epipericardial ridge together with the hypoglossal nerve. The long held view that the cervical sinus is obliterated by caudal growth of the hyoid arches to fuse with the cardiac elevation, excluding the succeeding arches from any part in the formation of the skin of the neck, has been criticized; an alternative view is that the sinus is reduced by gradual approximation of its walls from within outwards. It should be noted, however, that some contend that the surface course of the second groove persists as the curved submandibular *cervical flexure line.* Whatever the mechanism a smooth epidermal covering to the neck results with platysma (a second arch muscle), bounded both superficially and deep by superficial fascia, passing along the neck to the anterior thoracic wall.

## Pharyngeal Pouches

The first four pharyngeal pouches appear in sequence craniocaudally, and their endoderm approaches the ectoderm of the overlying pharyngeal grooves to form thin *closing membranes*

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(**3.139**, **140**). The blind recesses of the second, third and fourth pouches are prolonged dorsally and ventrally as angular, wing-like diverticula. From the fourth a diverticulum grows caudoventrally and is at first demarcated from the pouch by a groove in which may occur a transient fifth aortic arch artery. From this diverticulum a fifth pouch may develop and establish a connection with the ectoderm. The remainder of this diverticulum is the *ultimobranchial body*. This, together with the fourth pouch and the transitory fifth, when present, constitute the *caudal pharyngeal complex*. Its communication with the cavity of the pharynx is the *common pharyngobranchial duct.* The ultimobranchial body is almost a constant feature of vertebrate development (Watzka 1955). Its form in the human embryo, however, has been a matter of controversy. Apparently it is incorporated into the rest of the caudal pharyngeal complex and contributes to the development of the lateral thyroid rudiment (p. 177). Ultimobranchial bodies exist in the adults of many lower vertebrates and *calcitonin* has been isolated from such tissue (Copp et al 1967). There is thus a strong presumption that the parafollicular cells of the human thyroid gland, which are a source of calcitonin, are derived from ultimobranchial tissue. The further development of the endodermal derivatives of the pharyngeal pouches is intimately associated with that of the mouth, pharynx and larynx, and is considered with them (p. 176).

### *Rhombomeres, Hox Genes and Arch Development*

It has been seen in the above accounts that the cranial neural crest proliferates to form a significant mesenchymal population in the head, face and pharyngeal arches which controls the pattern of development of the face and arches, specifying the position of muscles, nerves and blood vessels. Experimental studies have shown that if first arch (mandibular) crest is grafted into the hyoid (second) arch, mandibular structures form, suggesting that the differentiation pattern of the second arch paraxial mesenchyme and surface ectoderm was redefined by the new crest mesenchyme (Noden 1988). Other experiments on tooth development (see above) illustrate the same phenomenon. These experiments, however, do not suggest whether the crest cells gain their patterning ability before leaving the neural plate or afterwards during their migration to the arches.

The neural crest cells migrate from the neural folds of the diencephalon, mesencephalon, metencephalon and myelencephalon; crest cells do not arise from the prosencephalic neural folds. At the time of crest migration the hindbrain (rhombencephalon) is composed of a repeating pattern of bulges, the rhombomeres (see p. 241), segmental units seen in the brains of all developing vertebrates. Single-cell marking experiments show that rhombomeres operate as distinct compartments with lineage restriction. There are eight rhombomeres identified in the hindbrain. Labelling of crest cells along the neural folds prior to migration has revealed a relationship between the sites of emergence of the crest cells and the rhombomeric epithelium (Lumsden et al 1991). Neural crest cells originate from three discontinuous levels and migrate ventrally in three distinct streams (**3.106A**). Crest cells from rhombomeres 1 and 2 contribute to the trigeminal ganglion and produce first arch mesenchyme, crest cells from rhombomere 4 contribute to the facial and vestibulo-acoustic ganglion and produce second arch mesenchyme, while crest cells from rhombomere 6 contribute to the superior petrosal ganglion and produce third arch mesenchyme. Two axial levels, rhombomeres 3 and 5, do not contribute to the emergent neural crest. However, crest cells migrating from rhombomeres 3 and 5 have been

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isolated in vitro, suggesting that in vivo the even-numbered rhombomeres may exert a dominant negative effect upon the odd numbered, suppressing neural crest production (Graham et al 1993 ). Such suppression, by segregating the crest into three distinct streams, may ensure the specific filling of each of the pharyngeal arches and the correct development of each of the individual cranial ganglia. The specification of the neural crest thus occurs before it migrates from the neural folds.

The axial homeobox genes, *Hox-a* and *Hox-b* (see p. 228), are expressed in the rhombomeres and in neural crest cells from the point of origin, during migration and after migration has ceased. Each pharyngeal arch expresses a different combination of *Hox* genes in a segment restricted manner (Hunt et al 1991; **3.106**, **148**). The exact relationship between *Hox* expression in the rhombomeres and later in the arches is not yet clear. For example, *Hox-b1* is delineated sharply in rhombomere 4 and later in arch 2; however, *Hox-b2* is expressed in all rhombomeres caudal from rhombomere 3, yet rhombomeres 3 and 5 do not produce migratory crest cells. Figure **3.148** shows the extent of *Hox* expression in the rhombomeres, the neural crest and the surface ectoderm.

Disruptions of the *Hox* genes cause failure of normal crest cell proliferation and migration, producing anomalies similar to human congenital disorders, for example DiGeorge's syndrome (see p. 228).

## Head Development and Evolution

The developmental mechanisms which operate within the trunk are different from those operating in the head: an observation which could be used to deduce that the head evolved by a different route from the other axial structures, using different cell populations which do not respond to, or differentiate earlier than, the inducers in the trunk region.

The vertebrate head is especially different from the 'cranial end' of its nearest relations, the cephalocaudates. They have, in common with vertebrates, segmented muscle blocks, a dorsal hollow nerve cord, gill slits and a notochord. However, they have no clear head, no obvious tripartite brain, no neural crest or ectodermal placodes, no paired sense organs or cranial ganglia. Amphioxus and other cephalocaudates may be considered to be distant relations of vertebrates; however, no link can be postulated that would demonstrate the gradual evolution of cephalization.

Until now, we have had few tools with which to examine the complexity and **comparative** nature of head development in extant species of vertebrates **and** cephalocaudates. The data now being generated by molecular biological studies on head development will have far-reaching effects and take much time to interpret.

A hypothesis of head evolution which is suggested by examination of the development of the head has been put forward by Gans and Northcutt (1983) and Northcutt and Gans (1983).

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They propose that the rostral part of the head, including the sense organs, prosencephalon, mesencephalon and surrounding skull, is derived from the neuroectoderm. Experimental studies have confirmed that the 'prechordal' skull, i.e. that part rostral to the notochord (Couly et al 1992), which surrounds the expanded rostral brain, is formed from neural crest, a population of neuroectoderm which invaginates between the neural tube and epidermal ectoderm, after neurulation. The neuroectoderm also gives rise to the sense organs via a series of ectodermal placodes, regions of neuroectoderm which do not separate from the epidermal ectoderm until the invaginated neural crest migrates beneath them. Between them, the cell populations produced by the neural crest and ectodermal placodes produce all of the sensory ganglia and sense organs within the head (the eyes, which are derived directly from the neural tube, are excluded from this group).

The neural crest also provides a new mesenchymal population which fulfils a role similar to that of the somatic and somatopleuric mesenchymes within the trunk. Specific interaction between the sclerotomal portion of the somite and the perinotochordal matrix promotes chondrogenesis around the notochord and neural tube resulting in the formation of the vertebrae. Condensations of somatopleuric mesenchyme within the limb have a chondrogenic fate when the cell density is high, and somatopleuric mesenchyme can be induced to follow this lineage in culture if the cells are arrested from migration and kept at high density. Neural crest cells, which never follow a chondrogenic pathway in the trunk, are able to differentiate into chondrocytes and other connective tissues in the head, and they are able to pattern the development of the facial primordia in the same manner as somatopleuric mesenchyme can pattern the limb. The mechanisms by which this occurs is not clear although the 'flypaper model', specifying the pattern of cranial chondrogenesis (Thorogood 1988), suggests that the neural crest responds to similar cues and in a similar manner as the sclerotomes (see p. 274).

It should be noted that the vertebrate head is formed not only by addition of neural crest in the rostral region but also by incorporation, in the caudal region, of an increasing number of vertebrae. The Agnatha have no vertebral contribution to the skull; the amphibians and selachians incorporate three occipital somites, whereas in the vertebrate skull all five occipital somites are included. This caudal enlargement contributes to the general increase in the volume of the skull around the expanding rhombencephalon at the same time as the crest-derived rostral portions of the skull surround the prosencephalon.

Mapping of the neural plate in the chick has shown that the prechordal skull is formed rostral to the adenohypophyseal placode from ectodermal and neural crest cells located in the neural folds. From rostral to caudal the neural folds produce the adenohypophysis (in the midline) and then on each side the olfactory ectodermal placode, the frontonasal ectoderm, the calvarial ectoderm and the cephalic neural crest (**3.145**). The ectoderm of the first pharyngeal arch is found lateral to the cephalic neural crest and it migrates rostrally and medially to contribute to the face (**3.145**). There is a neural crestfree gap over rhombomere 3 (see below) that separates presumptive frontonasal/ maxillary/mandibular cells from the second arch crest. Few or no crest cells are formed by neural folds at the level of the otic placode (rhombomere 5).

After neurulation, many structures present in the head of extant vertebrates are segmentally

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organized. For example, in the rhombencephalon there are ridges which divide the hindbrain into rhombomeres (**3.148**); subjacent paraxial mesenchyme is arranged as definitely segmented occipital somites and, more rostrally, possibly segmented somitomeres. More lateral and ventral locations contain the ectodermal placodes, the embryonic aortic arch arteries and the pharyngeal pouches. However, this segmentation is seen only caudal to the hypophysis, each side of the notochord. Thus the transient segmental nature of cephalic development is taking place in the 'ancestral' or 'old' head and brain. However, this paradigm is only partially supported by studies examining the expression of *Hox* genes in the developing brain.

*Hox* genes are expressed along the embryonic axis in invertebrate and vertebrate embryos. Recent cloning of *Hox* genes from cephalochordate embryos has shown an amphioxus *Hox* gene *AmphiHox-3* (Holland et al 1992) homologous to the mouse *Hox-2.7* gene. The rostral limit of expression of this gene in amphioxus is at the level of the 4/5 somite boundary at the neurula stage and at later stages within a spatially restricted domain of the developing nerve cord. Homologous gene expression in vertebrate embryos corresponds to the rhombomere 4/5 boundary. Holland et al propose that the structures expressing these genes in the two body plans are homologous. They suggest that this evidence supports the hypothesis that the vertebrate head evolved by elaboration and expansion of a pre-existing cranial region rather than by production of a new rostral portion. The utilization of extensive neural crest populations in the head may have resulted because it was a source of mesenchyme which could be modified and adapted without simultaneous reorganization of the trunk; the developmental flexibility of the crest population could promote an evolutionary flexibility and produce the diversity seen in vertebrate species today.

Noden (1991) adds a note of caution to the general interpretation of the developmental processes within the head, especially with the eruption of molecular biological applications for studying embryonic development. He recognizes that our present understanding of the morphology of development, of the patterns of cell movement, commitment and interactions which lead to the spatial assembly of complex arrays, is as yet inadequate to provide a basis for interpreting molecular analysis. The resolution of these challenges in the understanding of head development is awaited with interest.

## Appendicular Skeleton and Muscles

The appendicular skeleton and muscles arise from both paraxial mesenchyme (the epithelial somite) and lateral plate mesenchyme (somatopleuric).

### *Morphological Changes in the Limbs*

The limbs develop via a continual series of complex epithelial/ mesenchymal interactions initiated in the lateral body walls. The proliferating somatopleuric mesenchyme forms a ridge externally, ventrolateral to the somites, which extends caudally from the most caudal (sixth) pharyngeal arch, finally tapering towards the tail. Interaction of *specialized regions of the*

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*somatopleuric mesenchyme* with the overlying ectoderm gives rise to local, thickened regions of surface ectoderm and proliferation of the underlying mesenchyme; this specifies the position of the future limb buds. At the site of each putative limb the ectoderm forms a longitudinal ridge of high columnar epithelial cells, the *apical ectodermal ridge* (*AER*) (**3.149**, **150**). The AER and the underlying, specialized somatopleuric mesenchyme are termed the *progress zone*; this remains at the distal tip of the limb until the digits are formed. The progress zone controls the orientation and progression of limb development and specifies the position of the skeletal elements. The somatopleuric mesenchyme controls the specific developmental fate of the overlying ectoderm and within the limb becomes the skeletal and connective tissue elements. Precursor muscle cells and neurons migrate into the limb somewhat later. Consistent with the craniocaudal progression of development, the upper limb develops in advance of the lower. The earliest signs of limb development are seen in stage 12 (26 days) embryos. A ridge is visible along the lateral longitudinal axis of the body wall opposite somites 8–10, at the level of the entrance to the cranial intestinal portal; this is the upper limb bud. By stage 13 the lower limb bud is also visible.

The upper limb bud enlarges, protruding laterally from its elliptical base at the body wall as a flattened plate, with a curved border and an AER forming its distal tip; it also has initially equal and relatively flat *dorsal* and *ventral ectodermal surfaces*, and a somatopleuric mesenchymal core. For descriptive, experimental and conceptual purposes it has been necessary to define and name various 'axes', borders, surfaces and lines in relation to the bud (**3.150**). (However, some minor variation in *terminology* will be noted when human development is compared with basic tetrapod—amphibian, reptilian and also avian—development. Mechanisms, nevertheless, remain similar.) An imaginary line from the centre of the elliptical base of the bud, through the centre of its mesenchymal core, to the centre of the apical ectodermal ridge, defines the *proximodistal axis* of the bud (for long, in descriptive embryology, known simply as **the axis**). Named in relation to the latter, the limb border cranially placed is the *preaxial border* and that caudally placed is the *postaxial border*. (In tetrapods and birds, the latter are termed anterior and posterior borders, respectively; see below.) Any line passing through the limb bud from preaxial to postaxial border, (and orthogonal to the proximodistal axis) thus constitutes a *cranio-caudal axis*. The dorsal and ventral ectodermal surfaces thus clothe their respective aspects from preaxial to postaxial borders. Thus, any line passing from dorsal to ventral aspect (and orthogonal to both proximodistal and craniocaudal axes) constitutes a *dorsoventral axis*. (It should be noted here that the terms *dorsal* and *ventral axial lines* are to be used exclusively in relation to developing and definitive patterns of cutaneous innervation of the limbs and their associated levels of the trunk.

Early differential growth of parts of the limb bud result in two main changes to the originally symmetric axes of the limb:

(1) The dorsal aspect of the limb grows faster than the ventral; this causes the limb bud to curve around the body wall; the ventral surface of the limb which is closest to the body wall remains relatively flat but the dorsal surface bulges into the amniotic cavity; the originally laterally facing AER becomes increasingly directed ventrally.

(2) Slightly later the preaxial border grows faster than the postaxial, resulting in a further

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shift of the AER caudally rather than ventrally. These reorientations in the upper limb form the shoulder, arm and forearm; however, their effects cannot be seen until later (see below).

By stage 13 (28 days) the upper limb bud is curving ventrally while the lower limb bud is still directed laterally; in stage 14 embryos the preaxial border has started to lengthen in the upper limb but not yet in the lower. The upper limb at this stage is opposite the developing ventricles of the heart; the lower limb is closely associated with the wide umbilical cord. In stage 15 the upper limb can be subdivided into definite regions. The proximal portion of the limb still shows the dorsal bulge and ventral curve—this is the shoulder region and upper arm region; the next distal portion which was derived from the increase in the length of the preaxial border can now be identified as the forearm. The most distal portion is now expanded into a flattened hand plate.

At stage 16 the limbs appear much more substantial. The upper limb is sometimes close to the body wall and sometimes abducted; the lower limbs do not curve close to the body wall as the umbilical cord is very wide at this time. The hand plate has the first indications of digit rays and the lower limb has an early foot plate.

By stage 17 the upper limb has an elbow region and digit rays; in advanced members of this group the hand plate has a crenated rim indicating the beginning of tissue removal between the digits. The lower limb still has a flattened foot plate. Although a hip region can be seen there is no true knee as yet.

In stage 18 (44 days) embryos the foot plate has digit rays and there is further crenation of the hand plate between the digit rays. The lower limb appears to be flexed at the hip and abducted with the knee bent; this gives the appearance that the knee is facing laterally. There is very little skin of the thigh visible; the soles of the feet face the umbilical cord.

Changes during stages 19–23 are concerned with growth of the limbs and separation of the digits. The hands are now curving over the cardiac region. The distal phalangeal portions of the fingers enlarge at stage 21 forming the nail beds. This can be seen on the separated toes at stage 23. The feet can finally touch at stage 21 when the umbilical cord becomes proportionally smaller and the embryo larger.

## *Concepts of Limb Development*

Limb development may be conceptualized as resulting from a series of ectodermal/mesenchymal interactions (**3.150**, **151**). Such concepts are supported by experimental evidence from amphibian, avian and reptilian species which demonstrate a remarkable conservation of developmental processes. Chimeric experimentation has further revealed the specific fates of cell populations within the developing limb. The demonstration of conserved homeobox-containing genes in the developing limb (see below) may however require some reinterpretation of these concepts to reconcile the molecular model with the traditional model.

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## Progress Zone (AER-Mes)

The outgrowth of a limb bud is controlled by the apical ectodermal ridge (AER) and the *underlying somatopleuric mesenchyme*. The epithelium seems to control the developmental stage of the limb and the somatopleuric mesenchyme controls the type of limb, interpreting the temporal information from the AER in a proximodistal developmental progression. These two tissue arrangements form the *progress zone*, a region which is believed to be the site where assignments are made to cell populations in the limb. As cells leave the progress zone, their *proximal/distal value* becomes fixed. Once the mesenchyme has been assigned it specifies the developmental pattern of the overlying ectoderm.

The work of Zwilling (1972), Saunders et al (1976), Hinchcliffe and Johnson (1980) has provided much evidence of limb morphogenesis. The knowledge may be summarized as follows:

- The AER and underlying mesenchyme provide the orientating influence for limb outgrowth. Removal of the AER results in cessation of limb development; insertion of a second AER results in two axes of development: there is duplication of distal structures from the graft onwards.
- Replacement of the underlying mesenchyme with any other mesenchyme results in no limb development: only 'limb' mesenchyme will promote limb bud formation; however, replacement of upper limb mesenchyme with lower limb mesenchyme does support limb growth but leads to the development of leg structures. In addition the leg mesenchyme will pass information back to the local ectoderm causing appropriate leg feather (in chick) development. It is reasoned that the mesenchyme beneath the AER provides an 'AER maintenance factor' which is essential to the function of the ridge.

The *temporal* nature of the information passing from the AER to the underlying mesenchyme was illustrated in a series of experiments by Summerbell (1974). A graft of a young limb bud to an older one with the progress zone removed results in duplication of limb elements. Conversely a graft of an old progress zone onto the stump of a younger limb produces a limb with intermediate sections missing (see **3.150**). The progress zone behaves independently as if no communication concerning positional values travels in a proximodistal direction. As cells leave the progress zone their proximodistal values are specified (**3.150**).

In grafting experiments only whole limb bones develop. Eight states of the progress zone can be described: i.e. humerus, ulna-radius, carpals I, carpals II, metacarpals, phalanges I, phalanges II, phalanges III, each state taking approximately 8 hours. Summerbell and Lewis (1975) noted that the progress zone behaves like a clock whose ticks are cell-division cycles.

The precision with which skeletal growth occurs is often not appreciated. In calculating the growth in left limbs versus right limbs Summerbell et al (1973) concluded that the length of the left ulna of a limb did not vary by more than 5% of the length of the right.

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## Axes of the Limb

The three developmental axes can be identified in the developing limb bud by stage 13 (**3.150**). These are, as noted above, the *proximodistal*, the *dorsoventral* and the *craniocaudal* axes. Each of the three principal axes seem to be specified by different mechanisms. The *proximodistal axis*, as mentioned previously, is controlled by the *progress zone* (i.e. the AER and subjacent somatopleuric mesenchyme). The *craniocaudal axis* is controlled by a small population of mesenchymal cells on the postaxial border of the limb bud, some distance from the AER; this mesenchyme is termed the *zone of polarizing activity* (ZPA). The ZPA specifies digit five; further away from the ZPA digits four, three, two and one develop.

If the ZPA is grafted beneath an AER, duplication of the limb occurs from that time onwards. If the ZPA is grafted onto the preaxial border of the limb a duplicated distal portion grows with the orientation reversed (**3.150**). The *dorsoventral axis* of the limb appears to be controlled by the *ectoderm* of the limb. If the mesenchyme of a limb is removed and dissociated then repacked into the ectodermal sleeve, a limb will develop which has no anterior–posterior axis, i.e. the ZPA has been dispersed. However, the limb does have dorsal and ventral surfaces identified by the directions of the joints and position and type of hair.

## Early Skeletal Elements of the Limb

Formation of the cartilage elements of the limb has been suggested to be related to the shape of the limb and the conditions necessary for chondrogenesis. There is an *antichondrogenic zone* beneath the ectoderm of the limb which prevents chondrogenesis within the dermis and myogenic zones (p. 264). Foci of chondrogenesis occur in the **centre** of the limb bud where the cell density is highest, then the production of extracellular matrix by these cells encourages chondrogenic differentiation. In more distal portions of the limb, the limb bud widens forming first two centres of chondrogenesis, then later five centres. The AER is believed to control the width of the digital plate which in turn will reflect this width by the number of digits which develop. Zones on the cranial and caudal, or preaxial and postaxial borders of the limb which show preprogrammed cell death can be identified at the same time as the ZPA. These zones limit the length of the AER.

The experimentation concerning these zones was carried out in chick embryos. It is customary in anamniote embryology to refer to the craniocaudal axis as anteroposterior (with the human anterior and posterior surfaces being termed ventral and dorsal, respectively). This terminology has been retained for many amniote embryos, especially avian. Thus the special zones found on the pre- and postaxial borders of the limb are referred to, in the literature, as *anterior* and *posterior necrotic zones* (ANZ and PNZ). If the length of the AER becomes reduced then fewer digits will form—*oligodactyly*; if the AER is not reduced and becomes longer then more digits will form—*polydactyly*. This latter condition can permit the development of supernumerary digits on either the pre- or postaxial borders. There are other regions of cell death occurring between the digits which result in digital separation, but these occur later than the ANZ and PNZ. The cells between the digits are removed by macrophages. Note cells in the ANZ, PNZ and

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between the digits undergo apaptosis.

Most of the bones in the appendicular skeleton derive from somatopleuric mesenchyme. Within the upper limb, however, although the *clavicle* and *coracoid portion of the scapula* arise from somatopleuric mesenchyme, the *body and spine of the scapula* are believed to derive from the somites (Chevallier 1977). No recent studies yet dispute this finding.

*Prechondroblasts* are present in the upper limb at stage 13 and condensations of cartilage can be detected at stage 16 when the *humeral anlage* can be recognized. By stage 17, when the *radius* and *ulna* chondrify, the branched tips of the radial, median and ulnar nerves have migrated to the distal hand plate. The *carpal bones* chondrify at stage 18 when the hand plate shows notching of the digital rays. In the lower limb the *femur* and *tibia* have formed in cartilage and the sciatic nerve extends distally to the tibia by stage 18 (44 days).

The first evidence of bone formation is seen at the midpart of the diaphysis of long bones at 8 weeks. Vascular invasion of the cartilage matrix precedes the formation of a periosteal collar which extends proximally and distally until it reaches the future epiphyseal level where a *growth plate* will be established. By 10 weeks columns of chondrocytes can be seen at the epiphyseal level of most bones; however, only the lower end of the femur and upper end of the tibia develop ossification centres prior to birth (see p. 684). The pelvis forms from two hemipelves which each develop from one cartilaginous focus. Ossification of the pelvis commences with the ilium which undergoes endochondral ossification (similar to long bones) at 9.5 weeks.

## Development of Joints

Regions of developing cartilage are easily recognized in the developing limb as they have widely spaced cells surrounded by matrix. Between the developing skeletal elements the somatopleuric mesenchyme is more condensed forming plates of *interzonal mesenchyme* which mark the sites of future joints. Their development varies according to the type of joint formed.

In fibrous joints the interzone is converted into collagen, as the definitive connecting medium between the bones involved. In synchondroses it becomes (growth) cartilage of the modified hyaline type, whereas in symphyses the tissue is predominantly fibrocartilage, but retaining narrow para-osseous laminae of hyaline (growth) cartilage. The interzonal mesenchyme of developing synovial joints becomes trilaminar, due to the appearance of a more tenuous intermediate zone between two dense strata next to the cartilaginous ends of the skeletal elements of the region. As the skeletal elements chondrify and in part ossify, the dense strata of the interzonal mesenchyme also become cartilaginous and cavitation of the intermediate zone establishes the cavity or discontinuity of the joint. The loose mesenchyme around the cavity forms the synovial membrane and probably also gives rise to all other intra-articular structures, such as tendons, ligaments, discs and menisci. In joints containing discs or menisci and in compound articulations more than one cavity may appear initially, sometimes merging later into a complex single one. As development proceeds thickenings in the fibrous capsule can be recognized as the specializations peculiar to a particular joint. In some, however, such accessions

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to the fibrous capsule are derived from neighbouring tendons, muscles or cartilaginous elements.

Cavitation of the hip, shoulder and elbow joints has been reported at 7–8 weeks. The sacro-iliac joint can be recognized from 7 weeks, its development being slightly different from other synovial joints in that the development of the ilium is ahead of that of the sacrum. Uhthoff (1990) suggests that the initial stages in the process of cavitation of joints is independent of movements but that a full, true joint cavity can only form in the presence of movements.

Generally the literature suggests that all musculoskeletal elements are in their appropriate positions by 10 weeks. For a review of the literature concerning the chronology of events in human embryonic limbs consult O'Rahilly and Gardner (1975) and Uhthoff (1990).

### *Limb Musculature*

It is now well established that all limb muscle precursor cells originate from the somites (Jacob et al 1986). These precursor cells are committed at an early stage and can be identified in the lateral halves of the somites (Selleck & Stern 1991; Ordahl 1993). After the mesenchymal sclerotome cells have migrated from the epithelial somite the remaining dorsolateral portion is termed the *epithelial plate* of the somite (**3.131**). Cells from the cranial edge of this plate form the axial musculature whereas cells from the *ventrolateral edge* of those somites opposite limb buds migrate into the limb anlagen. Initially the cells migrate as single mesenchyme-like cells, then later in groups; they are surrounded by a non-random, structured network of extracellular fibrils. The migrating cells branch at their leading ends into filopodia which are in contact with the extracellular fibrils or with other cells. It is thought that the orientation of the extracellular fibrils may direct the migration of the cells. The precursor muscle cells are, however, not competent to produce limb muscles prior to their migration into the limb, and it is thought that the somito–somatopleural migration is a time when precursor myogenic cells acquire their responsiveness to the somatopleuric connective tissue.

The proliferation of the limb bud is controlled at the distal tip where the somatopleuric mesenchyme and the overlying ectoderm form the AER (p. 291). The myogenic cells colonize the limb bud in a *proximodistal direction only*, and never reach the most distal portion of the limb where there seems to be a distal boundary for the muscle cells. The speed of migration of myogenic cells into the limb is considered to be constant, since the border of invasion seems to lag behind as soon as the rate of elongation of the limb bud becomes more pronounced. Myogenic cells are still indifferent regarding their region-specific determination when they first enter the limb. Myogenic cells from a limb will, if grafted into brachial or pelvic somites, assume the myogenic potentialities of the somites and give rise to normal wing or leg musculature. The muscle cells, unlike the somatopleuric mesenchyme, have no 'limbness'. Further, the muscle pattern developed in the limb reflects the pattern of the skeletal elements; duplication or lack of digits is accompanied by the duplication or lack of the corresponding muscles.

Two subpopulations of myogenic cells can be discerned in the limb bud. In the early buds there are mainly *replicating presumptive myoblasts*, considered to be *premitotic*, whereas in later

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stages there are also *postmitotic myoblasts*. It is interesting that the invading myoblasts are still replicating; this may be a prerequisite for the formation of the considerable amount of skeletal muscular tissue which will develop in the limbs.

The first myogenic cells to arrive in the limb form the principal *dorsal* and *ventral premuscular masses*; it is thought that all classes of tetrapods begin limb muscle development with these blocks which produce all the skeletal muscle in the limb. The blocks of premuscle undergo a spatiotemporal sequence of divisions and subdivisions as the limb lengthens which leads to the individualization of about 19 muscles (**3.151**) in the upper limb and 14 muscles in the lower limb. The splitting process in the mouse commences at day 12 and is completed by day 17 (Kieny et al 1986). Small changes in the extracellular environment of myoblasts are believed to induce local fusion of some cells and thus create a gap which divides the muscle mass into two. In the upper limb, the premuscle masses first divide into three masses, the next division gives rise to the muscles attached to the carpus, and the final division produces the long muscles of the digits. A similar pattern is seen in the lower limb (**3.150**). Thus the patterning of the musculature of the limb is controlled by the somatopleuric mesenchyme.

The axial development of the limb, particularly that controlled by the ZPA, also affects the formation of individual muscles from the premuscular mass, as, if the somatopleuric mesenchyme is dissociated and repacked in an ectodermal sleeve prior to myoblast migration, the muscle masses remain unsplit.

Each anatomical muscle appears as a composite structure; the muscle cells and myosatellite cells are of somitic origin; the connective tissue envelopes and the tendons are of somatopleuric origin. The precise way in which the muscles are anchored to the developing bones by the tendons is not clear.

## *Embryonic Movements*

Embryonic *movements* are vital for development of the musculoskeletal system. As well as effects on the developing muscle they are necessary to align the trabeculae within the bones, the correct attachments of the tendons and the appropriate coiling of the constituent collagen fibres of the tendons. Simple movements of an extremity have been observed sporadically as early as the seventh week of gestation; combined movements of limb, trunk and head commence between 12 and 16 weeks of gestation. Fetal movements related to trunk and lower limb movements are perceived consistently by the mother from about 16 weeks gestation (quickening). Movements of the fetus are often slow, asymmetric twisting and stretching movements of the trunk and limbs, although there may be rapid, repetitive wide-amplitude limb movements. Movements of the embryo and fetus encourage normal skin growth and flexibility as well as the progressive maturation of the musculoskeletal system. It is noted that fetuses with dystrophies which prevent in utero movements develop webs of skin, *pterygia*, passing across the flexor aspects of joints which severely limit movements. A group of congenital disorders, collectively termed *multiple congenital contractures*, may result from genetic causes, limitations of embryonic and fetal joint mobility, or be secondary to muscular, connective tissue, skeletal or neurological abnormalities.

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These conditions may be recognized on prenatal ultrasound examination by the appearance of fixed, immobile limbs in bizarre positions, or by webbing in limb flexures. Specific syndromes, lethal multiple pterygium syndrome, and congenital muscular dystrophy have been described.

### *Hox Genes in the Developing Limb*

Study of the *Hox* gene clusters in limb development have provided an evolutionary explanation of the tetrapod condition and of the pentate form (Tabin 1992). Early prognathostomes had only a *ventrolateral skin fold* extending along the length of the body axis from which paired fins evolved. Migration of somatopleuric mesenchyme into separate regions of the ventrolateral skin fold specified the position of the early paired appendages. The segments of the body prior to limb development express various *Hox* genes in overlapping preaxial to postaxial domains. The site of limb formation could have a number of overlapping *Hox* gene domains present in the somatopleuric mesenchyme of the lateral body wall; evolution of the limb from this mesenchyme would result in elongation of these domains which then overlap, not like stripes but rather as nested sets, like Russian dolls.

The pelvic girdle is suggested to have developed first with the pectoral girdle reactivating the same genetic programme later, both limbs using *Hox-a* and *Hox-d* genes. There is molecular evidence which suggests that the pectoral girdle may have evolved from a modified branchial arch (Zanger 1981). The base of the branchial arches expresses *Hox-C*-6 which is also expressed in the extreme proximal, anterior region of the forelimb bud, but is not expressed in the hindlimb. This is of interest as chimera studies have shown that the scapula derives from somitic mesenchyme, while the clavicle, coracoid, sternum and pelvic girdles arise from somatopleuric mesenchyme (Gumpel-Pinot 1984).

Whereas both *Hox-a* and *Hox-d* are present in similar domains in the early limb bud, the *Hox-a* pattern shifts so that *Hox-a* genes show proximal/distal domains and *Hox-d* genes preaxial/postaxial domains. There are five genes in the *Hox-d* cluster which are expressed in the anterior/posterior axis. The nested arrangement of *Hox-d* genes means that the postaxial border of the limb has all *Hox-d* genes (*d*-13, *d*-12, *d*-11, *d*-10 and *d*-9) expressed; in the next anterior zone only four genes are expressed (*d*-12, *d*-11, *d*-10 and *d*-9), and so on until only *d*-9 is expressed. The five genes can specify five different types of digit. Polydactyly can be interpreted as duplication of an existing digit type but not the addition of a new type of digit. The genes do not however directly specify the digit structure, as the same *Hox* genes are expressed in both fore- and hindlimbs, and in homologous limbs of different species.

## Similarities in the Developmental Mechanisms of Facial Primordia and Limb Buds

The proximodistal outgrowth which constitute both the facial primordia and the limb buds are controlled by similar epithelial/mesenchymal interactions and it seems that the local environmental factors which, for example, control the outgrowths of either face or limb will

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support the other tissue type. Recombination experiments have shown that limb apical ectodermal ridge ectoderm can be maintained by mesenchyme from the three types of facial primordia, i.e. frontonasal, maxillary and mandibular. Of the three types of facial primordia in the chick, frontonasal and maxillary most resemble the limb in that they both contain rods of cartilage and undergo polarized outgrowth. Recombination of frontonasal mesenchyme and younger limb apical ectodermal ridge promoted the development of a cartilage rod in the primordium, forming an outgrowth which resembled an upper beak to the extent that an egg tooth developed. Thus the ectodermal signals from the limb were able to induce facial primordial development.

Reversed experiments, where limb mesenchyme was recombined with facial ectoderm also showed that supportive epithelial/mesenchymal interactions did occur. Interestingly both frontonasal epithelium and mandibular epithelium supported limb mesenchyme without any epithelial thickening, like an apical ectodermal ridge, which would normally be needed for proximodistal development of a limb. However, maxillary epithelium was not able to support limb outgrowth.

These experiments have demonstrated that the developmental signalling is similar but not identical in some facial primordia and the limb bud. One explanation for this could relate to the origin of the facial epithelium. The ectoderm covering the frontonasal process is derived from the neural fold of the prosencephalon (Couly & Le Douarin 1990), whereas the epithelium of the mandible and maxilla originates from ectoderm lateral to the neural folds (Couly & Le Douarin 1990). The neural crest mesenchyme within the facial primordia also has different origins, arising from different neural levels (see p. 286).

The development of an egg tooth provides an epithelial marker for distal differentiation in the frontonasal primordium and suggests that a progress zone operates within the frontonasal mesenchyme, similar to that in the limb. Similar patterns of expression of *MS*x1 and *MS*x2 are seen in both limb buds and facial primordia. The expression of these genes has been shown to depend on proximodistal position within the limb and this may prove to be the same in the facial primordia.

It will be interesting to see if other ectodermal/mesenchymal primordia such as those which develop around the urogenital membrane and form the external genitalia have similar or different signalling mechanisms.

## Skin and Appendages

Skin is developed from the surface ectoderm and its underlying mesenchyme. *Surface ectoderm* gives rise to the keratinizing general surface epidermis and its appendages, the pilosebaceous units, sudoriferous glands and nail units. It should also be noted that interactions between ectoderm and mesenchyme also give rise to the internal epithelium of the buccal cavity and the teeth (see p. 283) and the nasal epithelia (see p. 280). The more differentiated descendants

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of ectodermal cells are known as *keratinocytes* because their most characteristic contents are fibrous proteins called *keratins*, and also to distinguish them from *non-keratinocytes*, immigrant cells of different developmental origin which constitute an important component of the epithelial sheet formed by the keratinocytes, and with which they have a relationship which has been loosely termed 'symbiotic' (see p. 395). The non-keratinocytes are: the *melanocytes* derived from the neural crest; the *Langerhans cells* of bone-marrow origin; and *lymphocytes*. The *Merkel cell* is also usually classed as a non-keratinocyte, although it is being increasingly regarded as a modified keratinocyte.

The *dermis*, composed of irregular connective tissue and some of the connective tissue sheaths of peripheral nerves, derives from somatopleuric mesenchyme, for the limbs and trunk, possibly somitic mesenchyme over the epaxial musculature, and from neural crest in the head. Angiogenic mesenchyme gives rise to the blood vessels of the dermis. Nerves and associated Schwann cells, of neural tube and neural crest origin, enter and traverse the dermis to reach their peripheral terminations during development.

## Epidermis and Appendages

### *General (Interfollicular) Epidermis*

In the first 4–5 weeks, embryonic skin consists of a single layer of ectodermal cells overlying a mesenchyme containing cells of stellate dendritic appearance interconnected by slender processes, and sparsely distributed in a loosely arranged microfibrillar matrix (**3.152**). The interface between ectoderm (epidermis) and mesenchyme (dermis), known as the *Basement Membrane Zone* (BMZ), is an important site of mutual interactions upon which the maintenance of the two tissues depends both in prenatal and postnatal life (see below). Ectodermal cells, which characteristically contain glycogen deposits, contact each other at gap and tight junctions. The layer so formed soon develops into a bilaminar epithelium, the *epidermis*, when desmosomes also appear. The basal *germinative layer* gives rise to the definitive postnatal epidermis, and the superficial one to the *periderm*, a transient layer confined to fetal life. The periderm maintains itself and grows by the mitotic activity of its own cells, independent of those of the germinative layer, and expresses different keratin polypeptides. Originally flattened, the periderm cells increase in depth, with the central area containing the nucleus becoming elevated and projecting as a globular elevation towards the amniotic cavity (**3.153**). The plasma membrane develops numerous surface microvilli with an extraneous coat of glycosaminoglycans, and cytoplasmic vesicles become prominent deep to it. These developments reach a peak over the period 12–18 weeks, *at which time the periderm is a major source of the amniotic fluid* to which it may contribute glucose; it also has an absorptive function (Lane et al 1987). From about 20 weeks onwards, the globular protrusions become undermined and pinched off to float free in the amniotic fluid, and the now flattened periderm cells undergo a type of keratinization to form what is regarded as a temporary protective layer for the underlying developing epidermis proper, against an amniotic fluid of changing composition due to the accumulation of products of fetal renal excretion. Up to parturition, periderm squames continue to be cast off into the amniotic fluid, and they contribute to the *vernix caseosa*, a layer of cellular

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debris which covers the fetal skin at birth.

Proliferation in the germinative layer leads to a stratified appearance with successive layers of intermediate cells between it and the periderm. From an early stage, cells of all layers are packed with glycogen granules (**3.154**), presumably a source of energy during this early replicative stage of differentiation. Differentiation of these layers is not synchronous throughout all regions of the developing skin, being more advanced cranially than caudally, and on the body progressing from the midaxillary line ventrally. Reduction in glycogen content of the cells is associated with a shift towards biosynthetic activity connected with incipient *keratinization*, manifested by the presence of different enzymes and expression of keratins. Simple, low-weight keratins present from an early date are replaced by those of higher molecular weight associated with differentiation around 10–12 weeks, soon to be followed by profillagrin and fillagrin, and the appearance of keratohyalin granules among filamentous bundles of the uppermost intermediate layer cells at about 20 weeks. The first fully keratinized cells appear shortly afterward. By 24–26 weeks a definite stratum corneum exists in some areas, and by 30 weeks or so, apart from some lingering glycogen in intermediate cells, the interfollicular epidermis is essentially similar to that postnatally (see Breathnach 1971; Holbrook 1980, for further details).

## Melanocytes

Of neural crest origin, these are present in the bilaminar epidermis of cephalic regions as early as 8 weeks (Sagebiel & Odland 1972). By 12–14 weeks they can reach a density of 2300 per $mm^2$ reducing to 800 per $mm^2$ just before birth. Keratinocytes regulate the final ratio between themselves and melanocytes via growth factors, cell surface molecules and other signals (Scott & Haake 1991). Fetal melanocytes produce melanized melanosomes (see p. 389) and transfer them to keratinocytes, intrinsic activities clearly independent of ultra violet (u.v.) irradiation, and suggesting functions of melanin other than photoprotection.

## Langerhans Cells

These are of bone-marrow origin, are present in the epidermis by 5–6 weeks and are fully differentiated by 12–14 weeks (Breathnach & Wylie 1965). Their numbers increase at least partially by mitotic division in situ, but at 6 months are only 10–20% of those in the adult. It is not known if the Langerhans cell functions in immuno-surveillance in fetal skin.

## Merkel Cells

These appear in the glabrous epidermis of the palm and sole of the foot between 8 and 12 weeks (Moll et al 1986, 1990), and later in association with some hairs and with dermal axonal-Schwann-cell complexes. They are now thought to be modified keratinocytes rather than immigrants of neural crest origin (see p. 394).

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## Pilosebaceous Unit

Pilosebaceous units develop at about 9 weeks, first in the regions of the eyebrows, lips, and chin, and at progressively later stages elsewhere, proceeding caudally. The first rudiment is a crowding of cells in the basal layer of the epidermis—the *pregerm*. Further proliferation and elongation of the cells leads to a *hair germ*, which protrudes downwards into the mesenchyme where it becomes associated with an aggregation of cells, the primitive *dermal papilla*. With continued downward growth, in a slanted anteroposterior direction, the hair germ becomes a *hair peg*, and when its bulbous lower end envelops the dermal papilla it is known as a *bulbous peg* (**3.155**). At this stage three swellings appear on the posterior wall. The uppermost is the rudiment of the *apocrine gland* (present only in some follicles), the middle forms the *sebaceous gland* and the lower one is the *bulb*, to which the *arrector pili muscle* (arising from underlying mesenchyme) later becomes attached. The cells of the lowermost region of the bulb, the *matrix*, divide actively and produce a pointed *hair cone*, which grows upwards to canalize a developing *hair tract*, along which the fully formed hair, derived by further differentiation of cells advancing from matrix, reaches the surface.

Four successive stages of hair follicle development have been noted by Muller et al (1991). Stage I is characterized by invagination of the epidermis into the dermis which occurs prior to week 11 of gestation; stage II corresponds to the hair germ (see above) and has been described during weeks 13–15 of gestation. The appearance of the putative sebaceous gland from about week 16 is characteristic of stage III, and stage IV is reached when the dermal sheath and the sebaceous glands are differentiated and the hair passes through the skin surface, at about week 18 of gestation.

*Sebaceous glands* develop independently of hair follicles in the nostrils, eyelids (as tarsal glands) and in the anal region. *Apocrine sweat glands* are formed at the same time as eccrine glands (see below) and are at first distributed widely over the body; however, their number diminishes from 5 months' gestation resulting in the distribution seen in the adult (see p. 406). For further details of cellular events involved in ontogenetic differentiation of the hair and its sheaths, and of sebaceous and apocrine glands and the hair tract, see Sections 2 and 5. These processes are mirrored in the accelerated and compressed tempo of the differentiation of postnatal skin. Melanocytes are individually present at the hair-peg stage, and abundantly so and quite active in the bulbous peg. Langerhans cells have also been reported (Foster & Holbrook 1989).

*Developing hair follicles* are disposed in groups of three. Hairs produced prenatally are called *lanugo hairs*; they are short and downy, lack a medulla, and in certain parts of the body are arranged in a vortex-like manner into tracts. Late in pregnancy, lanugo hairs are replaced by *vellous hairs*, and these in turn by *intermediate hairs*, which are the predominant type until puberty. New follicles do not develop in postnatal skin.

## Eccrine Sweat Glands

Eccrine sweat glands are one type of sudoriferous gland. Sweat gland rudiments appear in the

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second and third months as cell buds associated with the primary epidermal ridges of the finger and toe pads of terminal digits. They elongate into the dermis and by 16 weeks the lower end begins to form the *secretory coil*, within which, by 22 weeks, *secretory* and *myoepithelial* cells are evident. The solid cord of cells connecting the coil to the epidermis becomes the *intradermal duct*, and the lumina of both are formed by dissolution of desmosomal contacts between the cells (Holbrook 1991). The *intraepidermal duct* is foreshadowed by a coiled column of concentrically arranged inner and outer cells, within which, by fusion of lysosomal vacuoles, a lumen is formed which opens on the surface at 22 weeks (Hashimoto et al 1966). As with hair follicles, no new eccrine glands develop postnatally. Sweating is said to be possible by 32 weeks, but clearly, has no functional significance in utero.

## Mammary Glands

Mammary glands are considered to be much modified sudoriferous glands and as such they are basically ingrowths from the ectoderm, which forms their ducts and alveoli, supported by vascularized connective tissue derived from the mesenchyme. In embryos of about the fifth or sixth week two ventral bands of thickened ectoderm, the *mammary ridges*, extend from axilla to the inguinal region, and in many mammals paired mammary glands develop at intervals along these ridges. In the human embryo the ridges are not prominent features, and only a single pair of glands develops in the pectoral region. The ridges disappear later in embryonic life, but before this the cranial third of each begins to show proliferation to form the two glandular rudiments. Supernumerary rudiments may form anywhere along the path of the mammary ridges and may develop into actual mammae or merely accessory or supernumerary nipples.

As each mammary primordium develops, its ectodermal ingrowth branches into 15–20 solid buds of ectoderm which will become the lactiferous ducts and their associated lobes of alveoli in the fully formed gland. These are surrounded by somatopleuric mesenchyme which forms the connective tissue, fat and vasculature and is invaded by the mammary nerves. By proliferation, elongation and further branching the alveoli are formed and the duct system defined. During the last two months of gestation the ducts become canalized and the epidermis at the point of original development of the gland forms a small *mammary pit*, into which the lactiferous tubules open. Perinatally the nipple is formed by mesenchymal proliferation. Should this fail the ducts open into shallow pits, a malformation known as inverted nipple. At birth the mammary glands are alike in their stage of development in both sexes, and in both some transient secretory activity may be observed, due presumably to circulating prolactin in the mother (Smith 1959). In males, thereafter, the mammary glands normally remain undeveloped; in females at puberty, in late pregnancy and during the period of lactation they undergo further, hormone dependent, developmental changes (pp. 418 et seq). For reviews of the prenatal histogenesis and ultrastructural appearances of mammary tissue consult Tobon and Salazar (1974): for postnatal reviews, pages 418 et seq.

## Epidermal Ridges

The epidermal ridges are foreshadowed as regularly spaced small down growths of epidermal cells which appear in finger and toe pads during the second and third months. They are known as

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*primary epidermal ridges*, separated by corresponding dermal ridges, and in the fifth month *secondary ridges* develop, the pattern becomes evident on the surface, and is finalized through further remodelling postnatally (Okajima 1975).

### *Nails*

Fields of proliferative ectoderm appear on the tips of the terminal segments of the digits; they progressively reach a dorsal position, where at about 9 weeks a flattened *nail field* limited by *proximal*, *distal*, and *lateral nail grooves* is apparent. The nail field ultimately forms the *nail bed*, and the primordium of the nail is formed of a wedge of cells which grows diagonally, proximally and deeply into the mesenchyme from the proximal groove towards the underlying terminal phalanx. The deeper cells of this wedge form the primordium of the *matrix* which gives rise to the *nail plate*; this emerges from under a, now proximal, nail fold at about 14 weeks to grow distally over an already keratinized nail bed. The nail matrix is usually considered to have dorsal and ventral (intermediate) components, but there are conflicting opinions as to the extent to which each contributes to the nail, both in ontogeny and postnatally; it is generally agreed that the ventral matrix contributes the major part. It has been claimed that the nail bed additionally contributes up to 20% of the postnatal nail plate (Johnson et al 1991), but embryological studies to date are not clear on this matter. Most texts state that keratohyalin is not involved in the keratinization of nail, but certainly, up to at least 16 weeks, the dorsal matrix granulosa cells which are contributing keratinized cells to the nail plate and *eponychium* (*cuticle*) contain typical keratohyalin granules, and the cells of the ventral matrix next to the nail plate contain single and compound granules similar to those present in granulosa cells of oral epithelia (Breathnach 1971). Similar granules have recently been reported by Picardo et al (1992) in matrix cells of postnatal human toenail.

At 20 weeks, the nail plate entirely covers the nail field (nail bed), now limited distally by a *distal ridge*, which, when the plate projects beyond the tip, becomes the *hyponychium* beneath it. At birth, the histology of the main nail unit components is similar to that postnatally (Zaias 1990); the nail is long and overhanging, and easily falls off during cleansing.

*Anomalous development of the epidermis* and its derivatives is relatively common. Excessive or diminished growth, or even complete absence, may affect sebaceous or sudoriferous glands and hair, either locally or generally. Similarly, the epidermis may be excessively pigmented (*melanism*) or lack melanocytes (*albinism*). Excessive keratinization leads to *ichthyosis*. A *naevus* or 'mole' is a locus of excessive pigmentation. Ectodermal dysplasia is a rare condition characterized by fine blond and scanty hair, reduced or absent eyelash and eyebrows. The skin has deficient sweat and sebaceous glands. Teeth are usually peg- or cone-shaped; absence of major salivary glands may occur.

## Dermis

The mesenchymal cells underlying the surface ectoderm and early bi- and trilaminar epidermis

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contact each other by slender processes (**3.156**) to form an intercommunicating network. They secrete a matrix which is rich in ions, water, and macromolecules, proteoglycan/glycosaminoglycans, fibronectin, collagenous proteins of various types and elastin. Further development of these intrinsic components involves the differentiation of individual cell types, fibroblasts, endothelial cells, mast cells, etc., and the assembly of matrix components into organized fibrillar structures—collagen fibres and elastic fibres. During embryogenesis, the matrix is heterogeneous with regard to its biochemical and macromolecular components, both in terms of relative composition, and local and temporal distributions and gradients, so that it is essential to think of matrix differentiation as well as cytodifferentiation during development. Progressive alterations in matrix components underlie many morphological dispositions. The main glycosaminoglycans of embryonic and fetal skin are glycuronic acid and dermatan sulfate. Collagens type I, III, V, and VI are distributed more or less uniformly regardless of fetal age, with some local concentrations of III and V, the levels of which are higher than in postnatal skin. Collagens type IV and VII are predominantly found in the Basement Membrane Zone.

The progressive morphological differentiation of the dermis involves its separation from the subcutis at about the third month; changes in composition and size of collagen fibrils and their organization into bundles amongst which cells become relatively fewer; downgrowth of epidermal appendages; the organization of nervous and vascular plexuses and the relatively late appearance of elastic networks. The papillary and reticular regions are said to be evident as early as 14 weeks, but the overall organization of the dermis continues postnatally (Holbrook 1991).

### Blood Vessels of the Dermis

The dermal vasculature is generally thought to be developed in situ by transformation of angiogenetic mesenchymal cells. Closed endothelial-lined channels containing nucleated red cells are present by 6 weeks underneath the ectoderm (Breathnach 1971) and by the eighth week are arranged in a single plane parallel to the epidermis to form ultimately the subpapillary plexus (Johnson & Holbrook 1989). A second deeper horizontal plexus is evident by 50–70 days, and both extend by budding as development proceeds. From these plexuses the final patterns of arterioles, venules and capillaries (see p. 399) develop, and they are established shortly after birth. Pericytes are also developed from mesenchymal cells.

### Lymphatic Vessels

These are formed by mesenchymal cells which become organized to enclose pools of proteinaceous fluid leaking from developing capillaries (Ryan 1991).

## Epithelial/Mesenchymal Interactions in Developing Skin

Epidermal/mesenchymal (dermal) interactions involving mutual inductive mechanisms are important during development and postnatally. They occur at the interface between the two, the *basement membrane zone* (BMZ), the development of which may be considered in

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morphological, biochemical, and immunological terms.

The basement membrane zone, at the ectodermal stage, consists of the basal plasma membrane of the ectoderm cell, paralleled on the cytoplasmic side by a skein of microfilaments, and beneath it, a layer (0.1–0.2 μm) of microfibrillar-amorphous material deposited by the cell (**3.157**). At the bilaminar stage, a definite continuous lamina densa is present, in the assembly of which fibronectin is involved, and it is separated from the basal plasma membrane by a lamina lucida traversed by loosely fibrillar material; similar filaments extend from the lamina densa into the mesenchymal matrix (**3.157**).

Hemidesmosomes begin to appear at 8 weeks as stratification starts, and anchoring fibrils at 9–10 weeks. By the end of the third month the basic morphology of the interfollicular BMZ is essentially similar to that postnatally (see p. 397). Immunocytochemical studies with monoclonal antibodies recognize the temporal onset of BMZ antigenic expression. For example, GB3 antigens (associated with hemidesmosomes) and laminin are shown to be present in the lamina lucida at 6 weeks, and LDA-1 antigen and collagen type IV in the lamina densa at the same time. Antigen LH7:2, associated with anchoring fibrils, is present at 8 weeks. Bullous pemphigoid antigen (hemidesmosomes) and antigens AF-1, AF-2 (anchoring fibrils) and KF 1 are expressed later, and the time of appearance of others is being regularly reported. These observations, combined with morphological ones, are of importance for prenatal diagnosis of genetically-determined diseases such as epidermolysis bullosa (Eady 1994).

The basal lamina provides a physical supporting substrate and attachment for the developing epidermis, and is thought to be selectively permeable to macromolecules and soluble factors regulating epidermal-dermal morphogenetic interactions. These have mainly been studied in other species, and in vitro (Sengel 1976; Woodley et al 1987), but it is likely that the general principles also apply in human development.

In the early stages of development the ectodermal/mesenchymal interactions contribute to the structuring of limb or facial primordia, e.g. the ectoderm promotes a chondrocyte free zone beneath it preventing chondrogenesis within the dermis and myogenic zones. Later, the dermis controls transformation of the ectoderm into epidermis, and regulates its basal–apical polarization, differentiation, and stratification, by maintaining controlled proliferation of the basal layer cells. The epidermis, in turn, induces the dermis to start morphogenesis. Complicated interactions are involved in the morphogenesis of the epidermal appendages, e.g. hairs, scales, feathers, as revealed by intra-class and inter-class dermal–epidermal recombinations. These have shown that the presence or absence of appendages is due to a regional property of the underlying dermis, which also determines their type, distribution and pattern. The epidermis determines the class-specific morphology of appendages, their cephalocaudal polarization, and the species-specific amino acid composition of keratins. For example in the chick, when mesenchyme from the thigh is inserted beneath ectoderm that covers the proximal portion of an embryonic wing, the wing ectoderm forms leg feathers. In fact combination of mouse mesenchyme (which would normally cause the overlying ectoderm to form hair) with chick corneal epithelium (which would normally become curved and transparent) results in the first

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stages of feather formation. The ectoderm constructs the typical appendage of avian skin being unable to 'interpret' the mouse mesenchyme instructions to form the mammalian appendages—hair (Wessells 1977). Many 'informative' and 'permissive' messages and signals between epidermal cells and dermal cells and matrix are involved in these overall interactions. Matrix macromolecules including some of those of the basement membrane zone mentioned above, i.e. fibronectin, integrins, cell adhesion molecules (cadherins), and soluble factors such as nerve growth factor, epidermal growth factor, retinoids and cyclic nucleotides have been suggested as mediators. There is evidence that calcium is involved as signal or messenger for some of the cell–substrate and cell–cell adhesive interactions involved (Fairley 1991). Similar interactions are also involved in wound healing and remodelling (see pp. 412, 416).

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